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ANTHRAQUINONE AND ANTHRONE SERIES

Part X. Some derivatives of benzanthrone and dibenzanthrone

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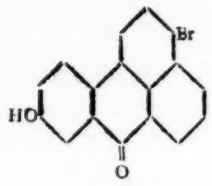
Received December 6, 1951

Mononitro and monohalogeno derivatives of benzanthrone with substituents in the anthrone ring cannot be prepared by direct substitution of benzanthrone, since the first substitution takes place in the meso-benzene ring. Benzanthrone derivatives of the former type are therefore prepared by the tedious route of cyclizing a suitably substituted phenyl α-naphthyl ketone,[1] or 1-phenylnaphthalene-2'-carboxylic acid,[2] or by the benzanthrone reaction on monosubstituted anthraquinones; by the second method a difficultly separable mixture usually results.[3] However, when 3-chloro or 3-bromobenzanthrone obtained by direct halogenation of benzanthrone is nitrated, the nitro group enters the 9-position. Reduction of the nitro derivative leads to 3-halogeno-9-aminobenzanthrone which can then be dehalogenated by methanolic caustic potash in the presence of palladized calcium carbonate activated by hydrazine hydrate. The synthesis of 9-aminobenzanthrone from 3-bromo-9-aminobenzanthrone in this manner has been reported earlier.[4]

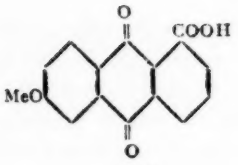
3-Bromo-9-aminobenzanthrone can be converted into 3-bromo-9-hydroxybenzanthrone (I) *via* the diazonium salt; (I) crystallizes in orange-red needles, m.p. 317–18°, the m.p. quoted in a patent[5] being 300°. Dehalogenation of (I) by means of hydrazine hydrate, methanolic caustic potash and palladized calcium carbonate gave 9-hydroxybenzanthrone (II), together with a small amount of 3:12-dihydroxydibenzanthrone. The formation of 3:12-diaminodibenzanthrone in the dehalogenation of 3-bromo-9-aminobenzanthrone has been recorded earlier.[4] The methylation of (I) and (II) gave 3-bromo-9-methoxybenzanthrone (III) and 9-methoxybenzanthrone (IV). Dehalogenation of (III) gave (IV) and 3:12-dimethoxydibenzanthrone (V). Unlike 16:17-dimethoxydibenzanthrone (Caledon Jade Green), (V) dyes a blue shade.

Chromic acid oxidation of (III) in acetic acid yielded 6-methoxyanthraquinone-1-carboxylic acid (VI).



(I)

(V)



(VI)

(VII)

The nitration of benzanthrone with nitric acid in acetic acid gave two isomeric mononitrobenzanthrones in proportions depending on the strength of the nitric acid. Pieroni[6] has reported that 3-nitrobenzanthrone, m.p. 246–47°, is formed by nitration of benzanthrone in acetic acid with 98% nitric acid, whereas a German patent[7] states that 2-nitrobenzanthrone, m.p. 298°, is formed when 88% nitric acid is added to a boiling solution of benzanthrone in acetic acid. Heilbron's Dictionary of Organic Compouds and Elsevier's Encyclopædia of Organic Chemistry do not list 2-nitrobenzanthrone. 2-Aminobenzanthrone is described in the patent literature as an intermediate for brown and grey dyes,[8] and Heilbron, *et al.*[9] have reported the synthesis of 2-chlorobenzanthrone from 2-aminobenzanthrone without indicating the source of the amine. Heilbron's Dictionary of Organic compounds does not list 2-aminobenzanthrone.

Nitration of benzanthrone in boiling glacial acetic acid gave a mixture of mononitrobenzanthrones from which a homogeneous mononitrobenzanthrone (VII), m.p. 302°, could be isolated in good yield by two crystallizations from nitrobenzene. This gave anthraquinone-1-carboxylic acid on oxidation and differed from 3-nitrobenzanthrone, m.p. 244–45°, showing that the nitro group is in the meso-benzene ring in the 1- or 2-position. Reduction of the nitro derivative gave an aminobenzanthrone, m.p. 234–35°, which on diazotization and boiling with sulphuric acid gave a hydroxybenzanthrone, m.p. 293–94° (decomp.). The latter proved to be identical with the hydroxybenzanthrone prepared by Badder,[10] since both the hydroxy derivatives gave 2-methoxybenzanthrone, m.p. 174°. Badder[10] prepared

the latter as follows: 3-acetamidobenzanthrone → 3-acetamido-2-nitrobenzanthrone → 2-nitro-3-aminobenzanthrone → 2- hydroxybenzanthrone → 2-methoxybenzanthrone. Since the methoxybenzanthrone prepared from the nitrobenzanthrone (VII) is the 2-derivative, (VII) is 2-nitrobenzanthrone, thus providing experimental proof of the patent claim.[7] Further, the aminobenzanthrone, m.p. 234–35°, obtained from this nitrobenzanthrone gave a chlorobenzanthrone, m.p. 191–92°, which is the m.p. cited for 2-chlorobenzanthrone.[9]

The residue left behind after 2-nitrobenzanthrone was separated from the crude reaction mixture also analysed for one nitro group, and oxidation gave anthraquinone-1-carboxylic acid, showing the absence of a nitrobenzanthrone with the substituent in the anthrone nucleus. Chromatographic analysis on alumina did not separate the isomeric nitrobenzanthrones. However, the amines obtained by reduction were separable by chromatographing a benzene solution on alumina. Two major bands developed; elution of the lower yellow band with benzene and concentration gave shining red needles of 2-aminobenzanthrone, m.p. 234–35°. The upper red band led to 3-aminobenzanthrone, m.p. 239–40°, unchanged by admixture with an authentic sample.[11]

Experimental

3-Bromo-9-hydroxybenzanthrone (I)

3-Bromo-9-aminobenzanthrone[4] (3 g.) was dissolved in sulphuric acid (30 c.c.), cooled to 0°, and treated with sodium nitrite (1·5 g.) during 30 mins. under stirring. On pouring into ice-water (600 c.c.) with stirring, the yellow diazonium sulphate that separated was filtered and washed with cold dilute sulphuric acid. The wet cake of the diazonium sulphate was added to boiling dilute sulphuric acid (conc. acid 400 c.c. and water 600 c.c.), when a copious yellow precipitate separated. It was filtered, washed with hot water until free from acid, and dried (2 g.; m.p. 305–10°). Two crystallizations from nitrobenzene gave shining orange-red needles, m.p. 317–18° (Found: C, 62·7; H, 2·8; Br, 24·8. $C_{17}H_9BrO_2$ requires C, 62·8; H, 2·8; Br, 24·6%). The substance gives a red solution in aqueous caustic soda and a bluish red solution in sulphuric acid.

3-Bromo-9-methoxybenzanthrone (III)

3-Bromo-9-hydroxybenzanthrone (1 g.) was suspended in 50% aqueous acetone (100 c.c.), and caustic soda solution (10%) and dimethyl sulphate were added in portions at 80° till the alkaline mixture was no longer red in colour. The yellow product that separated was collected, washed and dried

(1 g.; m.p. 198–200°). It crystallized from alcohol-tetrachloroethane in shining yellow needles, m.p. 201–02° (m.p. cited in a patent[5] is 196–97°) (Found: C, 63·4; H, 2·9; Br, 23·7. $C_{18}H_{11}BrO_2$ requires C, 63·7; H, 3·2; Br, 23·6%). The solution in sulphuric acid is bluish.

Reduction of 3-bromo-9-hydroxybenzanthrone (I)

3-Bromo-9-hydroxybenzanthrone (1 g.) was mixed with palladized calcium carbonate (0·8 g.) containing 1% palladium, methanol (20 c.c.), water (2 c.c.), caustic potash (2 g.) and an aqueous 85% solution of hydrazine hydrate (0·8 c.c.), and heated in a sealed tube at 155–60° for 90 mins. The reaction product was poured into water (100 c.c.), acidified with hydrochloric acid, boiled and filtered. The precipitate was collected, washed with water and dried; the greenish yellow product (0·67 g.) did not melt up to 360°. It was extracted with hot chlorobenzene till the extract was only faintly coloured. The chlorobenzene extract on concentration gave brownish orange needles of 9-hydroxybenzanthrone (II; 0·4 g.), m.p. 273–74° (Found: C, 82·8; H, 4·2. $C_{17}H_{10}O_2$ requires C, 82·9; H, 4·1%). It gives a red solution in aqueous alkali.

The residue (0·25 g), left after the chlorobenzene extraction, dyed a dull greenish shade, and gave 3:12-dihydroxydibenzanthrone as a blue powder on crystallization from nitrobenzene (Found: C, 83·9; H, 3·7. $C_{34}H_{16}O_4$ requires C, 83·6; H, 3·3%). It gives a violet vat with hydrosulphite and alkali.

Reduction of 3-bromo-9-methoxybenzanthrone (III)

Debromination was carried out as in the case of (I). The reaction product was worked up similarly, but the extraction was carried out with acetone. The extract, on concentration and dilution with water, gave 9-methoxybenzanthrone (IV) as yellow needles, m.p. 144–45° (Found: C, 85·3; H, 4·8. $C_{18}H_{12}O_2$ requires C, 85·0; H, 4·6%).

The acetone-insoluble portion, on crystallization from nitrobenzene, gave 3:12-dimethoxydibenzanthrone (V) as bluish violet needles (Found: C, 83·5; H, 3·6. $C_{36}H_{20}O_4$ requires C, 83·7; H, 3·9%). The product dyes cotton blue from an alkaline hydrosulphite vat which is violet in colour.

2-Nitrobenzanthrone (VII)

Benzanthrone (30 g.) was heated with acetic acid (450 c.c.) at 120° in an oil-bath, and a solution of nitric acid (*d.* 1·46; 21 c.c.) in acetic acid (150 c.c.) was added under stirring in 3 hours; stirring was continued at the same temperature for a further hour. The reaction mixture was cooled

and filtered; on washing the product with hot acetic acid, a yellow substance (23·6 g.) with a range of m.p. 260–90° was obtained. Two crystallizations from nitrobenzene gave fine yellow needles (11 g.), m.p. 302° (ref. 7, 298°) (Found: C, 73·9; H, 3·5; N, 5·3. $C_{17}H_9NO_3$ requires C, 74·1; H, 3·3; N, 5·1%). On oxidation with chromic acid in boiling acetic acid the substance gave anthraquinone-1-carboxylic acid, m.p. 290°.

2-Amino- and 3-aminobenzanthrones

The nitrobenzene mother liquors, on steam distillation, gave a crude mixture of nitrobenzanthrones (11 g.). This mixture (2 g.) was reduced with boiling methanolic sodium sulphide solution (2 g. fused sodium sulphide dissolved in 5 c.c. water and 35 c.c. methanol) under vigorous stirring for 4 hours. On filtering the cooled reaction mixture, the red product (1·3 g.) had m.p. 200–05°. Chromatography of a benzene solution of this substance on a column of alumina gave two main bands on developing with the same solvent. The lower yellow band was eluted with benzene, and concentration gave red needles of 2-aminobenzanthrone, m.p. 235°. From the upper red band, 3-aminobenzanthrone, m.p. 239–240°, was isolated.

2-Aminobenzanthrone

2-Nitrobenzanthrone (10 g.) was suspended in methanol (190 c.c.) and treated with fused sodium sulphide (10 g.) in water (10 c.c.). After stirring under reflux for 4 hours, the reaction mixture was cooled and filtered. The product, washed with water and dried (6·2 g.) had m.p. 225–30°. The amine was purified by dissolving in hot dilute hydrochloric acid and basifying. Crystallization from chlorobenzene gave shining red needles, m.p. 235° (Found: N, 5·8. $C_{17}H_{11}NO$ requiers N, 5·7%).

2-Acetamidobenzanthrone

2-Aminobenzanthrone (3 g.) was refluxed with a solution of acetic anhydride (25 c.c.) in acetic acid (25 c.c.) for 3 hours. On cooling, yellow needles separated (2·3 g.). Crystallization from chlorobenzene gave shining yellow needles, m.p. 272–73° (Found: N, 4·6. $C_{19}H_{13}NO_2$ requires N, 4·8%).

6-Methoxyanthraquinone-1-carboxylic acid (VI)

3-Bromo-9-methoxybenzanthrone (III; 0·65 g.) was refluxed with acetic acid (35 c.c.) and water (10 c.c.). Chromic acid (2·5 g.) was added in 2·5 hours and refluxing continued for 4 hours. On dilution with water (200 c.c.) and boiling with sulphuric acid (2 c.c.), a yellow flocculent precipitate separated, which was filtered, washed, and extracted with dilute ammonia,

Acidification of the ammoniacal solution gave the carboxylic acid (0·2 g.), which after two crystallizations from acetic acid was obtained as light yellow needles, m.p. 254–55° (Found: C, 67·9; H, 3·3. $C_{16}H_{10}O_6$ requires C, 68·1; H, 3·5%).

2-Hydroxybenzanthrone

A solution of sodium nitrite (0·15 g.) in sulphuric acid (3 c.c.) was added to an ice-cold solution of 2-aminobenzanthrone (0·3 g.) in sulphuric acid (4 c.c.) during 30 mins. After leaving for 2 hours the mixture was poured into ice-water (50 c.c.) and left for 1 hour. A test sample gave a deep red precipitate with alkaline β-naphthol. Excess of nitrous acid was destroyed with urea and the diazonium solution was poured into a boiling solution of sulphuric acid (60 c.c.) and water (40 c.c.). The yellow product that separated was filtered, washed and dried (0·3 g.). Purification by solution in dilute alkali (red solution) and reprecipitation with hydrochloric acid gave a bright yellow compound (0·2 g.), m.p. 280–85°. Crystallization from *o*-dichlorobenzene gave shining yellow needles, m.p. 293–94° (a patent[12] cites m.p. 287–88°) (Found: C, 82·7; H, 4·1. $C_{17}H_{10}O_2$ requires C, 82·9; H, 4·0%). Badder,[10] who has described a synthesis of 2-hydroxybenzanthrone, has not stated the m.p.

2-Methoxybenzanthrone

2-Hydroxybenzanthrone (0·13 g.) was dissolved in 5% caustic soda solution (50 c.c.) and warmed to 70°. Dimethyl sulphate was added in small portions under stirring till the alkaline mixture was no longer coloured. The yellow compound that separated was washed with dilute alkali, then with water and dried (0·13 g.; m.p. 165–70°). On crystallization from alcohol shining yellow needles, m.p. 173–74°, were obtained; Badder[10] quotes m.p. 174–74·5° (Found: C, 82·5; H, 4·6. Calc. for $C_{18}H_{12}O_2$: C, 82·1; H, 4·6%).

SUMMARY

Reduction of 3-bromo-9-hydroxybenzanthrone (I) and 3-bromo-9-methoxybenzanthrone (III) by palladized calcium carbonate and hydrazine gave 9-hydroxybenzanthrone (II) and 9-methoxybenzanthrone (IV) as the major products, together with 3:12-dihydroxydibenzanthrone and 3:12-dimethoxydibenzanthrone (V).

Nitration of benzanthrone in acetic acid and crystallization of the product from nitrobenzene gave a mononitrobenzanthrone (VII), which has been shown to be the 2-substituted derivative by reduction and conversion of the aminobenzanthrone to a hydroxybenzanthrone and methylation to

the known 2-methoxybenzanthrone.[10] The nitrobenzene mother liquors after separation of 2-nitrobenzanthrone contained 3-nitrobenzanthrone. 2-Nitro- and 3-nitrobenzanthrone in benzene solution do not separate on an alumina column, but the amines separate readily, 3-aminobenzanthrone being more strongly adsorbed.

We are indebted to the Council of Scientific and Industrial Research for a Research Fellowship awarded to one of us (P. N. P.), and to Mr. T. S. Gore for the microanalyses recorded in the paper.

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CHEMICAL EXAMINATION OF PLANT INSECTICIDES

Part VI. Root Bark of *Tephrosia lanceolata* Grah.

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Received December 24, 1951

Tephrosia lanceolata Grah. is a small herbaceous plant belonging to the family Leguminosæ and has the following special characters which distinguish it from other Tephrosia species. The plant is sub-erect, with the stems coloured distinctly red when fresh. Branches are green on the under-surface and reddish brown on the upper surface. The leaflets are lanceolate and their under-face is silvery. The flowers are small with the corolla thinly silky and red. The pods are more than an inch long and contain 5–6 seeds.

Most of the other Tephrosia species which have been examined in the past have been found to be toxic to fish and to yield crystalline compounds.[1] The root bark of *T. lanceolata* (for the identification we are indebted to the Systematic Botanist, Agricultural Research Institute, Coimbatore) was therefore examined by us for possible action as a fish poison. For our experiments we employed the fresh-water fish *Haplochilus panchax* available at Waltair. It was found that when an alcoholic extract of the powder was added to water in which the fish were kept, they showed toxic symptoms and died. For the quantitative study of toxicity we have employed the 'turning point', a term introduced by Krishnaswamy and Seshadri,[2] as the criterion. The concentration and time reported in this communication have reference to the turning point as defined by these authors. Tested in this manner the root bark powder exhibited definite toxicity in 19 minutes in a concentration of 66 p.p.m. or 6 minutes in a concentration of 166 p.p.m. It was therefore subjected to detailed investigation and the results are described below.

The plants required for the study were collected from sandy tracts round about Waltair where they grow wild and in fair profusion. The material was obtained in two seasons: (1) September 1950 and (2) May and June 1951. Part of the material collected in 1950 was subjected to extraction with ether and then with chloroform, while another part was extracted directly with chloroform. The results obtained in these experiments are,

or convenience, described after the results obtained with the 1951 collection which constituted the bulk of our material.

The powdered air-dried bark (1951 sample) was extracted with chloroform in the cold and the extract was concentrated to small bulk. Since no solid separated even after standing for a number of days at a low temperature, the solvent was completely removed under reduced pressure and the residue taken up in ether. The ether solution was extracted with aqueous potash thus effecting a separation into alkali-soluble and alkali-insoluble fractions. From the ether solution representing the alkali-insoluble fraction, a pale yellow crystalline solid, "Substance I", separated spontaneously. The mother-liquor from the crystals was concentrated to a syrupy consistency and treated with benzene. "Substance II" separated at this stage in a very small yield. The filtrate from Substance II, when further diluted with benzene and kept at a low temperature, yielded a crystalline solid, which could be separated into Substance I and another entity, "Substance III". The amorphous residue recovered from the mother-liquor was toxic to fish (5 p.p.m. toxic in 22 min.).

From the alkali-soluble portion no crystalline compound could be obtained. The amorphous substance was found to be toxic to fish (66 p.p.m. toxic in 11 min.).

The marc remaining after chloroform extraction was extracted with warm alcohol, and the extract concentrated to small bulk. Since no solid separated even after standing in the ice chest for a number of days, it was concentrated further and the residue poured with stirring into a large excess of water. The precipitated resinous material was divided into alkali-soluble and alkali-insoluble fractions by maceration with dilute aqueous alkali. The alkali-soluble portion was recovered by neutralising the filtered alkaline solution. It could be divided into two portions, chloroform-soluble and chloroform-insoluble. Thus the alcohol extract was obtained in three fractions, alkali-insoluble, alkali- and chloroform-soluble, and alkali-soluble but chloroform-insoluble. All of them were resinous and could not be crystallised from organic solvents. In fish tests with these fractions purging was observed in all the three cases, but only the first and the second were toxic (12 min. in a concentration of 10 p.p.m. or 6 min. in a concentration of 60 p.p.m. in the first case, and 140 min. in a concentration of 200 p.p.m. or 24 min. in a concentration of 320 p.p.m. in the second case). Attempts to prepare crystalline acetyl derivatives or to obtain crystalline degradation products by boiling with 10% aqueous potash yielded negative results in all the three cases.

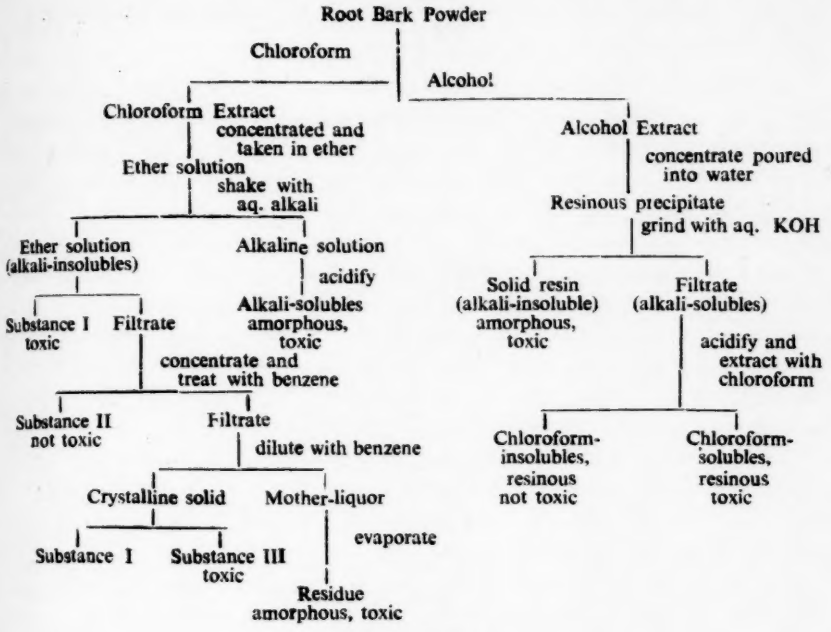
Thus from the 1951 sample three crystalline compounds, Substances I, II and III were isolated.

From the 1950 collection a 400 g. sample was extracted with chloroform and the chloroform extract examined in a manner analogous to that described under the 1951 sample. However only Substance I could be obtained as the crystalline component and it was isolated from the alkali-insoluble portion. Substances II and III could not be obtained, even though, as a result of the findings on the 1951 sample, they were specially looked for. Another 400 g. sample of the 1950 collection was extracted in stages, being first exhausted with ether before extraction with chloroform. The ether extract yielded a small amount of Substance I directly on concentration. The subsequent chloroform extract was worked up in the same manner as already described. Again only Substance I was obtained under the alkali-insoluble part and Substances II and III could not be got even after careful searching. The difference between the 1950 and 1951 samples with reference to the crystalline components is probably to be attributed to seasonal variations.

In view of the results obtained with the 1951 sample the marc of the 1950 sample remaining after the chloroform extraction was not subjected to extraction with alcohol.

Substance I (pale yellow needles from alcohol) melted at 187–88° and had the probable formula $C_{22}H_{22}O_4$. It was toxic to fish (17 min. in a concentration of 33 p.p.m.). Substance II (nodules), even after repeated recrystallisation, did not have a sharp melting point, 135–42°. It was not toxic to fish. In view of the very small amount available for study and the unsatisfactory nature of its purity it has not been analysed. Substance III (colourless woolly needles from alcohol) melted (with evolution of water vapour) at about 122°, then solidified and melted again at 147–49°, probable formula $C_{23}H_{14}O_4$, $2H_2O$. The anhydrous substance melted directly at 147–49° without previous sintering. It was much less toxic to fish than Substance I (12 hours in a concentration of 33 p.p.m.; the toxicity test could not be performed with higher concentrations because of the very low solubility of the substance in water). All the three substances seem to be new. The results of detailed examination of Substances I and III will be described in a future communication.

The course of the extraction and isolation of the different fractions is schematically represented below:



Experimental

3·8 Kg. of the powdered air-dried root bark (1951 collection) was extracted with chloroform by maceration in the cold (5 times—each time 15 litres). The marc was then spread out on a tray to remove remnants of chloroform and extracted with methylated spirit in a metal Soxhlet extractor (only 1·5 kg. of the marc was employed for this purpose) until the extraction was complete. The extracted powder was discarded.

Chloroform Extract.—The combined chloroform extracts were concentrated to 500 c.c. and the concentrate left in the ice-chest for 15 days. No solid separated out. The solvent was removed completely under reduced pressure and the residue taken up in 1 litre of ether and left in the ice-chest. A small amount of resin sticking to the sides was removed after 2 days by decantation. The clear ether solution was rapidly extracted with 5% aqueous potassium hydroxide (4 × 100 c.c.) and then washed with water till neutral. Even during the washing with water a crystalline solid began to separate out. It was filtered after 24 hours and washed with ether (crude Substance I, yield 4·6 g.). The filtrate was dried over sodium sulphate, concentrated to 300 c.c. and left in the ice-chest. No solid separated even after 15 days. The solution was concentrated to a syrupy consistency and treated with

benzene (200 c.c.). A small quantity of solid that separated immediately was filtered off (impure Substance II, yield 0·1 g.). The filtrate was diluted with more benzene (200 c.c.) and left in the ice-chest for 10 days. The solid that had separated was filtered, washed with benzene and dried (impure mixture of Substances I and III, 4 g.). The semi-solid residue obtained by evaporation of the mother-liquor amounted to 200 g.

The alkaline solution was neutralised with 1 : 1 hydrochloric acid and the solution extracted with ether (5 × 100 c.c.). The ether solutions were washed neutral with water, dried over sodium sulphate, concentrated to 200 c.c. and left in the ice-chest for a month. No solid separated even then. It was next concentrated to a syrupy consistency and treated with benzene (100 c.c.) and left in the ice-chest. Even then no crystalline material could be obtained. Semi-solid residue obtained by evaporation of the solution weighed 45 g.

Substance I.—The impure substance after repeated crystallisation from rectified spirit yielded pure Substance I as pale yellow needles melting at 187–88° (3 g.). It was sparingly soluble in ether, moderately soluble in hot alcohol and readily soluble in chloroform and benzene. It did not give any colour with ferric chloride. With conc. sulphuric acid a yellow solution was obtained. In the Durham test it turned deep orange with nitric acid, but did not develop a blue colour with ammonia. It did not give any colour with acetic anhydride and sulphuric acid in chloroform solution (Liebermann-Burchard reaction) or on reduction with magnesium and hydrochloric acid (Found: C, 75·41; H, 6·40%; $C_{22}H_{22}O_4$ requires C, 75·43; H, 6·29%).

Substance II.—This was sparingly soluble in most of the organic solvents (ether, chloroform, acetone). It was moderately soluble in hot ethyl alcohol from which it was crystallised. Even after repeated crystallisations it could be obtained only as nodules and the melting point was not sharp, 135–42°. In the Durham test no colour was observed. Conc. sulphuric acid did not give any colour nor did reduction with magnesium and hydrochloric acid.

Substance III.—The impure mixture of I and III already referred to was first crystallised a few times from benzene-alcohol (1 : 1) whereby a sample melting indefinitely at 125–32° was obtained. This was then fractionally crystallised from rectified spirit. The less soluble fraction consisted of pale yellow needles melting at 187–88° (Substance I), while the more soluble consisted of colourless woolly needles melting (with evolution of water vapour) at about 122°, then solidifying and melting again at 147–49° (Substance III, yield 2 g.). Substance III was readily soluble in acetone, chloroform, warm benzene and hot alcohol. It did not give a colour with

ferric chloride or sulphuric acid or nitric acid. Reduction with magnesium and hydrochloric acid yielded only a pale yellow colour but not red. After drying in vacuum at 70° for one hour the substance melted at 147–49° without any previous sintering (Found: loss on drying at 110° *in vacuo*, 10·29%; $C_{23}H_{14}O_4$, $2H_2O$ requires loss on drying 9·23%. Found on the anhydrous sample: C, 77·91; H, 3·95%; $C_{23}H_{14}O_4$ requires C, 77·96, H, 3·96%.

Alcohol Extract (from 1·5 kg. of marc.).—The alcoholic extract in the Soxhlet extractor was concentrated to 500 c.c. and left in the ice-chest for 15 days. No solid separated. It was concentrated further to 100 c.c. and poured with stirring into 1 litre of water. The precipitate was allowed to settle overnight and the supernatant liquid was decanted off. The resinous solid was extracted with 4 × 50 c.c. of 2% aq. potassium hydroxide. The alkali-insoluble part was washed free from alkali and purified further by redissolving in alcohol and reprecipitation by pouring the solution into water (yield, 2 g. of reddish brown resin).

The alkaline solution was shaken with ether and the residue from the ether solution added to the alkali-insolubles. The aqueous layer was then neutralised with 1 : 1 hydrochloric acid, whereupon a certain amount of precipitate separated. The entire mixture was extracted with 5 × 100 c.c. of chloroform. A good amount of the material could not be extracted with chloroform and this remained as a layer floating in the aqueous phase. The chloroform solutions were collected, washed neutral, dried over sodium sulphate, concentrated to 50 c.c. and left in the ice-chest. No solid separated even after a number of days. The solution was poured into 500 c.c. of petroleum ether. The precipitate obtained was purified by re-solution in chloroform and reprecipitation by dilution with petroleum ether. The filtered and dried solid (resinous) amounted to 2·3 g.

The chloroform-insoluble portion which floated in the aqueous phase (see above para) was separated by filtration, washed free from acid and dried (4 g., resinous).

As already mentioned in the introduction, neither the alkali-insoluble part of the alcohol extract, nor the chloroform-soluble part of the corresponding alkali-solubles nor the chloroform-insoluble part of the alkali-solubles could be crystallised from organic solvents. For toxicity tests with these fractions (results described in the theoretical part), 10 c.c. of a 5% solution of gelatin per 500 c.c. of water was employed as stabiliser. The acetylation and hydrolysis with aq. potash were carried out on the following lines.

250 mg. of the resin was refluxed with 1 g. of fused sodium acetate and 5 c.c. of acetic anhydride for 4 hours on an oil-bath maintained at 140°. The mixture was cooled, poured into 200 c.c. of water with stirring and left overnight. The precipitated material was filtered and washed with water. It could not be crystallised from any solvents.

For alkaline hydrolysis 1 g. of the resin was boiled under reflux with 80 c.c. of 10% aq. potash for one hour. After cooling, the solution was neutralised with 1 : 1 hydrochloric acid and the mixture containing some precipitate was repeatedly extracted with ether. A small amount of material remained insoluble in ether in every case. It was filtered, washed and dried and examined separately. From the ether-soluble part of the fission products acidic and phenolic fractions were separated by shaking the ether solution with 5% aq. sodium carbonate and 5% aq. sodium hydroxide respectively. The remaining ether solution was washed with water, dried over sodium sulphate and evaporated to give the neutral products. The sodium carbonate and sodium hydroxide solutions were separately acidified and extracted with ether and the ether solutions evaporated to give the acidic and phenolic fractions. Crystalline compounds could not be obtained in any one of these experiments.

SUMMARY

The isolation of three crystalline substances from the chloroform extract of the root bark of *Tephrosia lanceolata* Grah. is described. All of them seem to be new. These substances, as also certain amorphous fractions of the chloroform extract and the subsequent alcoholic extract have been studied for their toxicity towards fish and the results are recorded. Attempts to prepare crystalline acetyl derivatives or to obtain crystalline degradation products by alkaline hydrolysis of the different fractions of the alcoholic extract have yielded negative results.

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EFFECT OF PYRIDINE ON THE REDUCTION OF OXYGEN IN PULSATING FIELDS

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Introduction

The use of pulsating fields for the study of electrode kinetics indicated by Breyer and Gutman[1] has been adopted in a modified form in this laboratory and a number of systems have already been studied.[2, 3, 4] The present work deals with the behaviour of oxygen in pulsating fields and the influence of pyridine.

Experimental

The experimental arrangement is the same as the one used previously.[3, 4] Normal sodium hydroxide is used as the supporting electrolyte and the potential applied through the mercury pool. The D.C. potential—A.C. current curves obtained at different frequencies of A.C. for normal sodium hydroxide containing air are represented in Fig. 1. Similar curves for

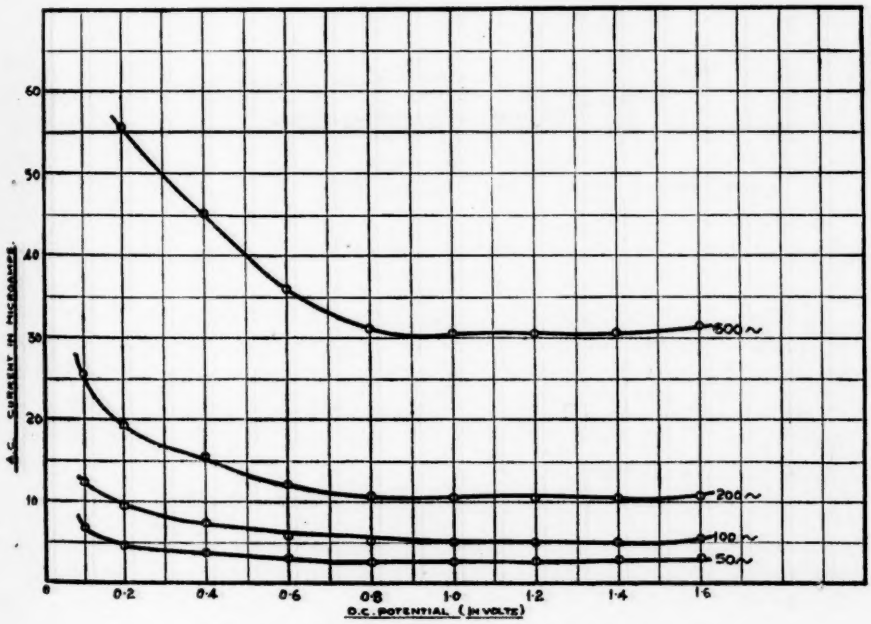

Fig. 1. Behaviour of Normal Sodium Hydroxide in Pulsating Fields.

normal sodium hydroxide containing varying concentrations (volume per cent.) of pyridine are given in Fig. 2. Fig. 3 represents the curves for sodium hydroxide containing 1% pyridine at different frequencies.

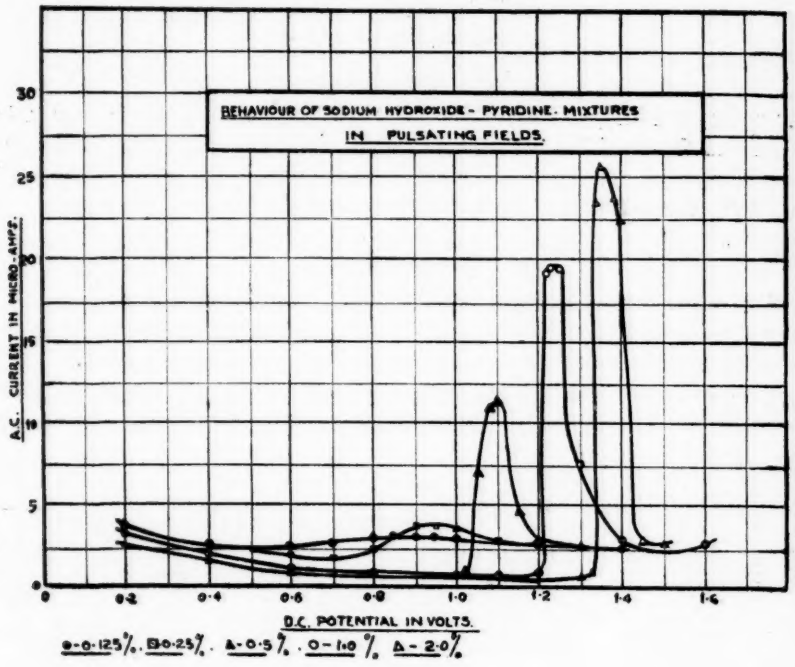

FIG. 2.

DISCUSSION

When oxygen is subjected to polarographic reduction, two waves are obtained. The first wave is got in the neighbourhood of $-0{\cdot}1$ V with reference to saturated calomel electrode or $+0{\cdot}04$ V with reference to NaOH (N) . HgO (S)/Hg electrode which corresponds to the pool in our experiments. This wave is due to the reduction of oxygen to hydrogen peroxide by the reaction

$$O_2 + H_2O + 2e \rightarrow H_2O_2 + 2OH^-.$$

The second drawn out wave with half-wave potential of about $-0{\cdot}9$ V (S.C.E.) corresponds to the reduction of hydrogen peroxide to hydroxyl ion by the equation

$$H_2O_2 + 2e \rightarrow 2OH^-.$$

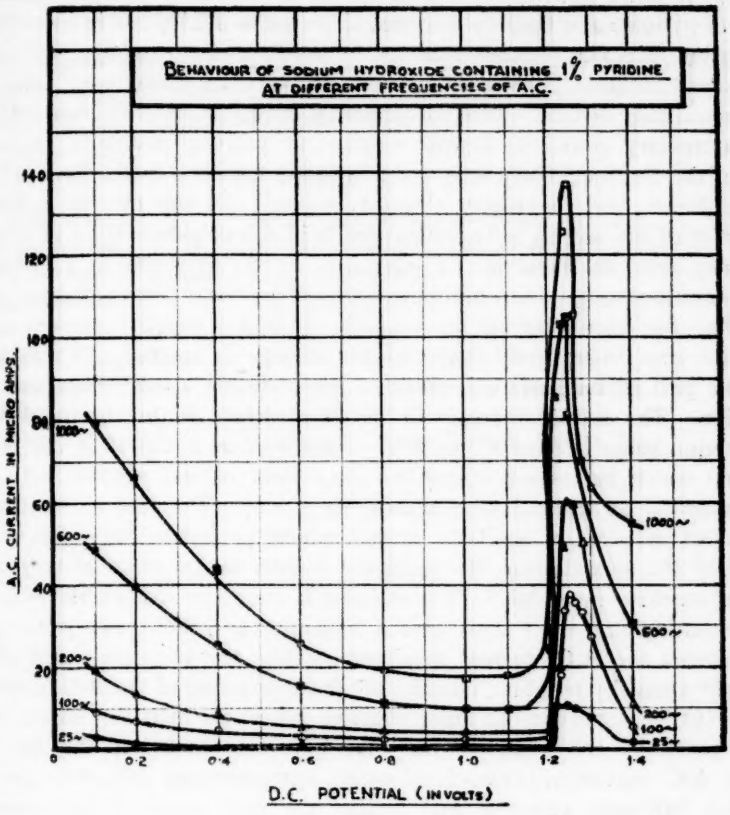

FIG. 3

We have confirmed the occurrence of these two polarographic waves with solutions used in these experiments. It is interesting to note that there are no corresponding maxima obtained in the D.C. potential—A.C. current curves. These maxima are hereafter referred to as "B.-G. maximum" or "Breyer-Gutman maximum" so as to distinguish from polarographic maxima. This can be interpreted on the basis of the theory put forth earlier[4] that the reduction of oxygen is so slow that it does not follow the pulsations even when the frequency is as low as 50 cycles per second. It is interesting to find that addition of pyridine at once causes the B-G maximum corresponding to the second wave of the polarographic reduction of oxygen to appear. It is of further interest to note that the B-G maximum is shifted

to more negative potentials. The maximum is very sharp at high concentrations of pyridine and becomes rounded as we go to 0·25% and lower. These interesting facts can be accounted for by the following reasoning. Pyridine in alkaline medium is more or less hydrophobic and tends to get adsorbed at the mercury surface. This adsorption is strong in the neighbourhood of electrocapillary zero. At highly negative or positive potentials on either side of the electrocapillary zero, the adsorption falls due to the water dipoles being drawn into the highly charged surface. As the normal half-wave potential of the second wave for oxygen is in the neighbourhood of electrocapillary zero, the reduction of oxygen is largely prevented as it is unable to penetrate through the coherent pyridine film. Such hindrance by adsorption has also been observed by Keilin.[5] At higher negative potentials, the pyridine gets progressively desorbed and a stage is reached when a considerable part of the surface is more or less suddenly available for electrode reaction. The sudden increase in the free surface is due to the heat of adsorption being affected by the applied potential as discussed in one of our papers[6] which makes the adsorption vary more or less exponentially with the potential as referred to electrocapillary zero. The rate of reaction as expressed per unit of available surface would be indeed very high at the point of the maximum as the reduction is now taking place at very much higher negative potentials.[7] The reaction is therefore easily able to follow the pulsating field even at as high a frequency as 1,000 cycles per second, and causes the A.C. current maximum. Thus the two interesting effects brought about by pyridine, namely, (*a*) the appearance of the B-G maximum and 1 (*b*) its occurrence at more negative potentials than the normal half-wave potential, are accounted for. We have also confirmed that the shift of the A.C. maximum is associated with a corresponding shift of the polarographic half-wave potential and further the high speed of the electrode reaction is confirmed by the increasing steepness of the polarographic waves occurring at the higher negative potentials. The addition of camphor causing a shift in the hydrogen peroxide wave making it steep and consequently well measurable[8] is a phenomenon very much analogous to the present one and has to be accounted for similarly. It is to be noted that pyridine does not cause the B-G maximum corresponding to the first wave to appear. This is perhaps due to the position of the maximum being shifted to more positive potentials due to the adsorbed film of pyridine disappearing only at high positive potentials and its getting rounded off due to the diminution in electrode reaction velocity at the low potentials. Thus the B-G technique provides a new and effective method for the study of the

effect of surface-active substances on electrode kinetics. It is proposed to extend the investigation to other surface-active substances.*

The authors wish to thank Prof. J. M. Saha, Director, Indian Institute of Sugar Technology, Kanpur, for his kind interest. Thanks are also due to the Uttar Pradesh Scientific Research Committee for a grant which made this work possible.

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* The question as to how far the observed B-G maxima are caused by mere changes in capacity caused by the pulsating field and how far by electrode reaction is a matter for future investigation.

ACTION OF ACIDIC REAGENTS ON ANHYDRO SUGARS

Part I. The Action of Acidic Reagents on 4 : 6-Benzylidene-2 : 3-Anhydro-α-Methyalloside and 4 : 6-Benzylidene-2 : 3-Anhydro-α-Methylmannoside

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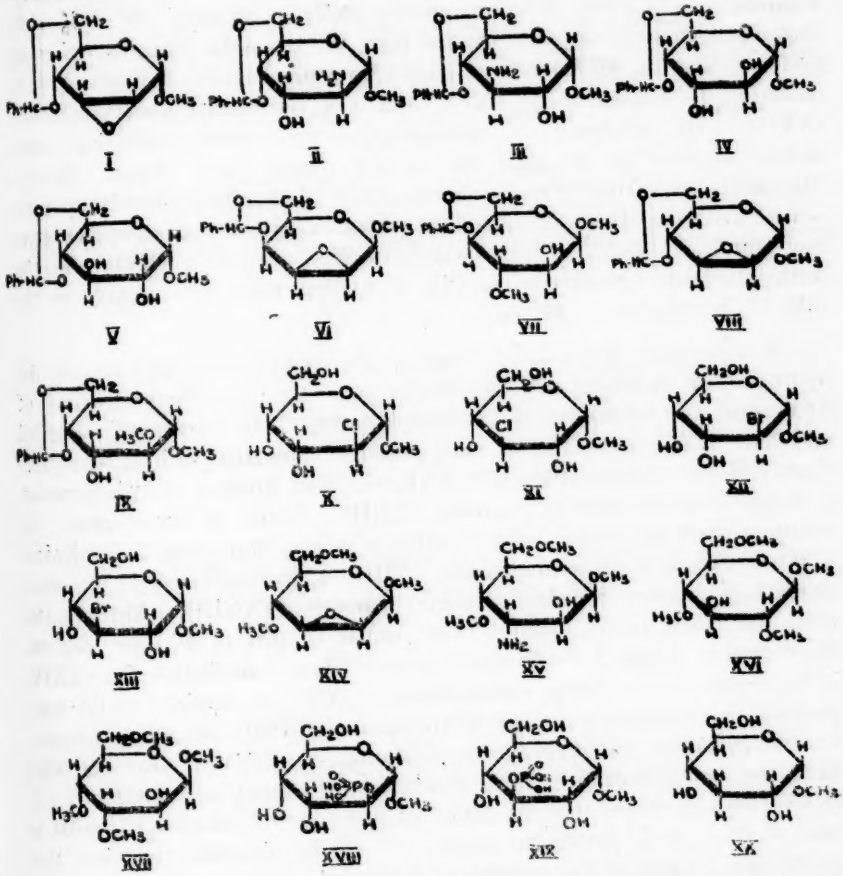
(Communicated by Dr. K. S. Gururaja Doss, F.A.SC.)

THE action of alkaline reagents on the ethylene oxide ring of anhydro sugars has been studied extensively but not much is known about the action of acidic reagents on such rings. In the scission of epoxide ring whether by alkali or by acid, invariably two isomeric products are produced and more often than not, one of them is present in a larger quantity. In view of the previous results on the scission of anhydro rings by alkaline reagents, and on the basis of their studies on the action of hydrochloric and hydrobromic acids on 4: 6-benzylidene-2: 3-anhydro α-methylalloside, Newth, Overend and Wiggins[1] put forward a rule governing such scissions. According to this rule, in the opening of 2: 3-ethylene oxide anhydro rings in sugars with alkaline reagents, if the ring lies above the plane of the sugar ring then the C—O bond furthest from the glycosidic group suffers the most extensive scission, whereas if the anhydro ring lies below the plane of the sugar ring, then it is the C—O bond nearest to the glycosidic group which breaks to the greater extent. The acidic reagents have a similar effect but in reverse direction at least in one case examined by them, *viz.*, the action of hydrochloric and hydrobromic acids on 4: 6-benzylidene-2: 3-anhydro α-methylalloside. Thus, Peat and Wiggins[2] studied the action of ammonia on 4: 6-benzylidene-2: 3-anhydro α-methylalloside (I) and found that 2-amino-4: 6-benzylidene α-methylaltroside (II) and 3-amino-4: 6-benzylidene α-methylglucoside (III) were obtained in 80% and 10% yields respectively. Richtmyer and Hudson,[3] on opening the anhydro ring of 4: 6-benzylidene-2: 3-anhydro α-methylalloside (I) with potassium hydroxide obtained 4: 6-benzylidene α-methylaltroside (IV) and 4: 6-benzylidene α-methylglucoside (V) in 84% and 7% yields respectively. The ethylene oxide anhydro ring of 4: 6-benzylidene-2: 3-anhydro β-methyltaloside (VI) on being opened with sodium methoxide[4] yielded mainly 4: 6-benzylidene-3-methyl β-methyl

idoside (VII). Again, by the action of sodium methoxide on 4: 6-benzylidene-2: 3-anhydro α-methylmannoside (VIII), Robertson and Griffith[6] obtained 4: 6-benzylidene-2-methylaltroside (IX) as the main product. Thus, in the above cases of ethylene oxide anhydro ring scissions by alkaline reagents, the rules of Newth, *et al.* (*loc. cit.*) are followed.



By the action of hydrochloric acid on 4: 6-benzylidene-2: 3-anhydro α-methylalloside (I), Newth, *et al.* (*loc. cit.*) obtained a mixture of 2-deoxy-2-chloro α-methylaltroside (X) and 3-deoxy-3-chloro α-methylglucoside (XI) with the latter predominating. The difference in the quantities of the two isomers, however, is not pronounced. Similarly, the products obtained by the action of hydrobromic acid were the corresponding bromo derivatives.

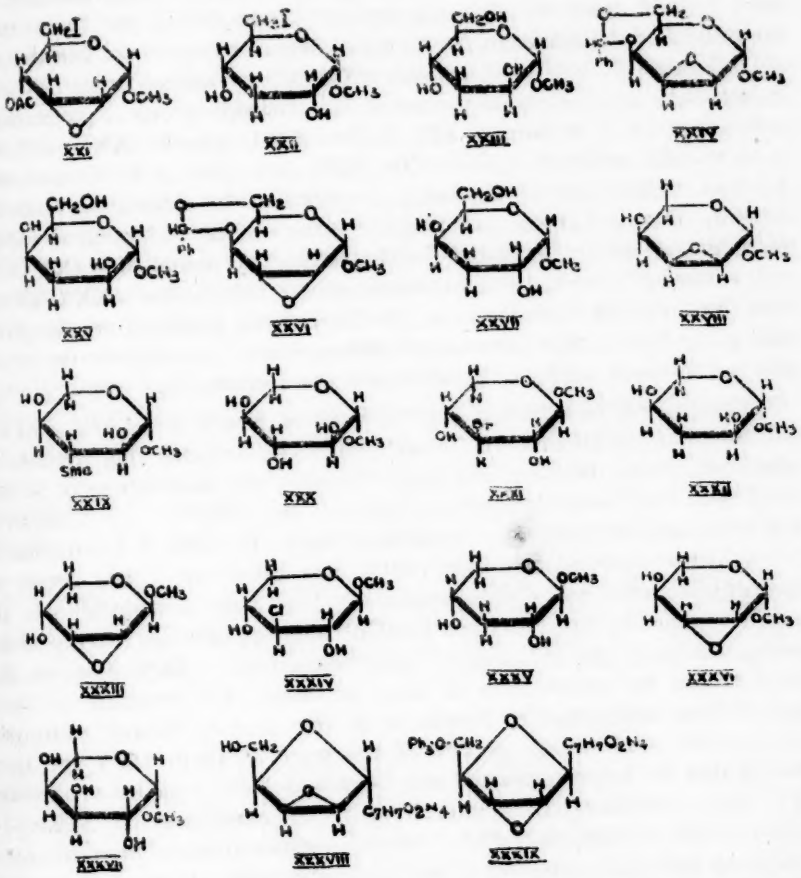
2-deoxy-2-bromo α-methylaltroside (XII) and 3-deoxy-3-bromo α-methyl glucoside (XIII), the latter being produced to the lagrer extent.

The rules of Newth, *et al.* (*loc. cit.*), however, are not applicable in some of the cases of anhydro ring scissions. Thus, in the case of 2:3-anhydro-4:6-dimethyl β-methylmannoside (XIV), whereas with ammonia,[6] 3-deoxy-3-amino 4:6-dimethyl β-methylaltroside (XV) is obtained in 90% yield showing that the C—O bond furthest from the glycosidic group is broken up preferentially, the products of scission with sodium methoxide[7], *viz.*, 2:4:6-trimethyl β-methylglucoside (XVI) and 3:4:6-trimethyl β-methylaltroside (XVII), were produced in equimolecular proportions indicating equal facility of cleavage on each side of the oxygen ring. Again, Harvey, Michalski and Todd[8] have shown that 2:3-anhydro-4:6-benzylidene α-methylalloside (I) on being treated with dibenzylhydrogenphosphate gives four times as much of the methylaltroside-2-phosphate (XVIII) as the methylglucoside-3-phosphate (XIX), a finding which is contrary to the rules of Newth, *et al.* (*loc. cit.*).

It is not clear again, in what manner the ring scissions take place in the hydrogenation reactions of 2:3-anhydro sugars, *e.g.*, on hydrogenation of 2:3-anhydro-4:6-benzylidene α-methylalloside[9] (I), 3-deoxy α-methyl glucoside (XX) is formed as the main product. Similarly, with 2:3-anhydro-4-acetyl-6-iodo α-methylalloside[10] (XXI), the main product of hydrogenation is 6-iodo-3-deoxy α-methylglucoside (XXII). Thus, in these cases, the action of hydrogen is very similar to that of acids. But, when 2:3-anhydro-4:6-benzylidene α-methylmannoside (VIII) is hydrogenated,[11] the main product of reaction is 3-deoxy α-methylmannoside (XXIII). Thus, in this case, the action of hydrogen is very similar to that of alkaline reagents. Furthermore, when 2:3-anhydro-4:6-benzylidene α-methyltaloside (XXIV) is hydrogenated,[12] 3-deoxy α-methylidoside (XXV) is obtained as the main product and which is analogous to the action of alkalis on anhydro sugars. 4:6-Benzylidene-2:3-anhydro α-methylguloside[3] (XXVI), on the other hand, on being hydrogenated[13], gives mainly 3-deoxy α-methylgalactoside (XXVII) which means that the action of hydrogen in this case is similar to that of acids on 2:3-anhydro sugars. It will be observed, therefore, that in the above cases of the opening of 2:3-ethylene oxide anhydro rings by hydrogenation, the C—O bond furthest from the glycosidic group, *i.e.*, the bond between C_{-3} and O breaks to a larger extent irrespective of the fact whether the epoxide ring is above or below the plane of the sugar ring.

Having considered the action of acidic and alkaline reagents and hydrogen on 2:3-anhydro rings in aldohexopyranosides, it would be of

interest to study the action of these reagents on such rings in aldopentopyranosides since the nature of the sugar ring in the latter and the aldohexopyranosides is almost similar. Thus, when 2:3-anhydro β-methyl-L-riboside (XXVIII) is treated with sodium thiomethoxide[14] and potassium

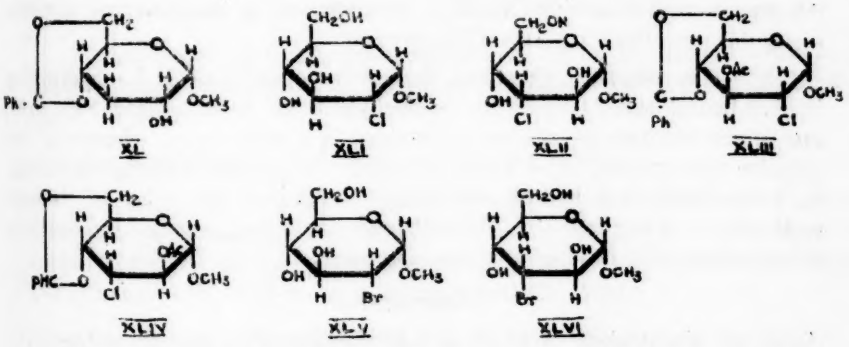


hydroxide,[15] 3-thiomethyl β-methyl-L-xylopyranoside (XXIX) and β-methyl-L-xyloside (XXX) are produced to the larger extent respectively. Similarly, with hydrobromic acid[16] and hydrogen,[14] the main products are 3-deoxy-3-bromo β-methyl-D-xyloside (XXXI) (from 2:3-anhydro-β-methyl-D-riboside- and 3-deoxy β-methyl-L-xyloside (XXXII) (from 2:3-anhydro-β-methyl-L) riboside) respectively. Similarly, Allerton and Overend[17] have shown that in

the cleavage of the 2:3-anhydro ring of β-methyl-2:3-anhydro-D-riboside (XXXIII) with hydrochloric acid and with lithium aluminium hydride, β-methyl-3-deoxy-3-chloro-D-xyloside (XXXIV) and β-methyl-3-deoxy-D-xyloside (XXXV) are respectively produced as the main products. Thus, in all cases of anhydro ring scission in 2:3-anhydro ribopyranosides, the bond furthest from the glycosidic group is ruptured to the larger extent irrespective of the nature of the reagent. When, however, the anhydro ring of 2:3-anhydro β-methyl-L-lyxoside (XXXVI) is opened with potassium hydroxide,[15] the C—O bond nearest to the glycosidic group is preferentially ruptured giving a mixture of 65% of β-methyl-L-xyloside (XXX) and 35% of β-methyl-L-arabinoside (XXXVII) which fact again is at variance with the rules of Newth, *et al.* (*loc. cit.*), if applied to the aldopentopyranosides. Recently, Davoll, Lythgoe and Trippett[18] have studied the action of sodium thiomethoxide on 2′:3′-anhydro-7-α-D-lyxofuranosyl theophylline (XXXVIII) and 5′-trityl-2′:3′-anhydro-7-β-D-ribofuranosyl theophylline (XXXIX) and have shown that in both the cases, the C—O bond furthest from the glycosidic group breaks to a larger extent although the 2:3-anhydro ring in one case is above and in the other below the plane of the sugar ring.

Thus, it will be observed that the rules of Newth, *et al.* (*loc. cit.*) are not applicable in all cases of 2:3-ethylene oxide anhydro ring scissions in sugars and it was, therefore, with a view to extend the observations of Newth, *et al.* (*loc. cit.*) that the present work on the anhydro ring scissions in 4:6-benzylidene-2:3-anhydro α-methylalloside (I) and 4:6-benzylidene-2:3-anhydro α-methylmannoside (VIII) was taken up. The action of hydrochloric acid on 4:6-benzylidene-2:3-anhydro α-methylalloside (I) was first studied by Robertson and Dunlop[19] and they obtained two crystalline products of m.p. 161–162° and 137–138° respectively. They, however, did not establish the constitution of these products. The structure of these products was established by Newth, *et al.* (*loc. cit.*) by the acid hydrolysis and acetone condensation of one of the products (m.p. 161°) and they showed that the latter compound was 2-deoxy-2-chloro α-methyl-D-altroside (X). The compound having m.p. 136-138° was designated as 3-deoxy-3-chloro α-methyl-D-glucoside (XI). We have also arrived at the same conclusion by periodate oxidation of the two compounds. It can be seen that when 4:6-benzylidene-2:3-anhydro α-methylalloside is treated with hydrochloric acid, the formation of only two isomeric chlorohydrins (X and XI) is possible. From the examination of the formulæ (X and XI), it is evident that the compound of the formula (X) should react with one mole of periodate since it possesses a pair of α-glycol groups, whereas the compound (XI) should not at all react with periodate. The compound having m.p. 162°

reacts with one mole of periodate and therefore, it must be designated as 2-deoxy-2-chloro α-methylaltropyranoside (X). The other compound (m.p. 137–138°) did not react with periodate and hence it must be designated as 3-deoxy-3-chloro α-methyl-D-glucopyranoside (XI). An additional proof of the fact that the compound having m.p. 137–138° is represented by the formula (XI) has been furnished by hydrogenation reaction. When it was hydrogenated in presence of palladium catalyst (on barium sulphate) in dilute potassium hydroxide, the halogen atom was smoothly replaced by hydrogen atom with the liberation of one mole of hydrochloric acid to give 3-deoxy α-methylglucoside (XX) which was isolated as 4: 6-benzylidene-3-deoxy α-methylglucopyranoside (XL), the m.p. of which was not depressed when mixed with an authentic specimen of 3-deoxy-4: 6-benzylidene α-methyl glucoside prepared by the method of Prins (*loc. cit.*). In one of the experiments, a small amount of 4: 6-benzylidene-2: 3-anhydro α-methyl alloside (I) was obtained presumably by the action of alkali present during hydrogenation in conformity with the results of Robertson and Dunlop (*loc. cit.*). Newth, *et al.* (*loc. cit.*) have recorded that although 3-deoxy-3-chloro-α-methylglucoside (XI) was obtained in a larger quantity than 2-deoxy-2-chloro α-methylaltroside (X), the difference in the amounts of the two isomers was not so pronounced. It has now been shown that the crystalline reaction mixture of 3-deoxy-3-chloro α-methylglucoside and 2-deoxy-2-chloro α-methylaltroside contains 75% of the former isomer and 25% of the latter, since the mixture consumed 0·25 mol./mol. of sodium periodate.



4: 6-Benzylidene-2: 3-anhydro α-methylmannoside (VIII) on being treated with acetonic hydrochloric acid gives a syrup which does not crystallise and consequently the separation of the two chlorohexosides, *viz.*, 2-deoxy-2-chloro α-methylglucoside (XLI) and 3-deoxy-3-chloro α-methylaltroside (XLII) expected to be formed on opening of the anhydro ring, could not be

effected. It will be observed that 2-deoxy-2-chloro α-methylglucoside (XLI) has a pair of contiguous hydroxyl groups and therefore should consume a mole of sodium periodate, whereas 3-deoxy-3-chloro α-methylaltroside (XLII) should not at all react since it does not possess a free 1:2-glycol system. Thus, by the oxidation of the syrup containing the two chloro hexosides, it has been found that 2-deoxy-2-chloro α-methyl glucoside and 3-deoxy-3-chloro α-methylaltroside are present in the proportion of 56·1: 43·9. The syrup was condensed with benzaldehyde but the benzylidenated product too did not crystallise. On acetylation of the latter, however, a crystalline product was obtained which on recrystallisation gave a substance having a sharp m.p. of 126°, and the analysis of which corresponded to 4:6-benzylidene-chloro-acetyl α-methylhexoside. This compound must, therefore, be either 2-deoxy-2-chloro-3-acetyl-4:6-benzylidene α-methyl glucoside (XLIII) or 3-deoxy-3-chloro-2-acetyl-4:6-benzylidene α-methyl altroside (XLIV). An attempt was made to replace the chlorine atom of the compound with hydrogen so as to get a deoxy sugar which could be identified, but the hydrogenation could not be effected.

Similar experiments on the opening of anhydro ring of 4:6-benzylidene-2:3-anhydro α-methylmannoside (VIII) by hydrobromic acid were carried out but in this case too, the resultant product did not crystallise and was obtained in the form of a syrup. Estimation of isomeric bromo-methylhexosides in the syrup by periodate oxidation showed the presence of 52·4% of 2-deoxy-2-bromo α-methylglucoside (XLV) and 47·6% of 3-deoxy-3-bromo α-methylaltroside (XLVI). Benzylidenation and acetylation of the syrup did not afford any crystalline product.

It will be observed, therefore, that in the opening of 2:3-anhydro ring in 4:6-benzylidene-2:3-anhydro α-methylalloside with hydrochloric acid, one of the products of scission is obtained in a large excess whereas in the anhydro ring opening of 4:6-benzylidene-2:3-anhydro α-methylmannoside by hydrochloric and hydrobromide acids, although the rules of Newth, *et al.* (*loc. cit.*) are followed, the difference in the amounts of the isomeric halogenohexosides produced, is not appreciable.

EXPERIMENTAL

Action of Hydrochloric Acid on 4:6-*Benzylidene* 2:3-*Anhydro* α-*Methyl-D-Allopyranoside*

4:6-Benzylidene-2:3-anhydro α-methyl-D-allopyranoside (9 g.) prepared according to the method of Richtmyer and Hudson (*loc. cit.*) was dissolved in acetone (900 c.c.) and hydrochloric acid (33·3 c.c., 2N) added. The solution was boiled under reflux for 3 hours. The acid was neutralised with

barium carbonate and the solution filtered. The precipitate was washed with acetone, the combined filtrate and washings were evaporated until free from acetone and the aqueous solution was extracted with ether to remove benzaldehyde. The aqueous solution was then evaporated to dryness under reduced pressure (bath temp. 40°) and the residue extracted with warm acetone; the acetone solution on being evaporated gave a crystalline solid (5·5 g.). The product was dissolved in minimum quantity of ethanol which on filtration and cooling deposited a crystalline substance (Fraction A). The mother liquor on treatment with light petroleum gave a second Fraction (B). Fraction (A) was crystallised repeatedly from ethanol until the melting point was constant. In this way an α-methyl chlorohexoside, m.p. 161–162° was obtained which was shown to be 2-deoxy-2-chloro α-methylaltroside. (Found: C, 39·35; H, 6·5. $C_7H_{13}O_5Cl$ requires C, 39·53; H, 6·16%.) Fraction (B) was crystallised repeatedly from petrol ether-alcohol, then from acetone-ether until the melting point was constant (m.p. 138°) and was proved to be 3-chloro α-methylglucoside. (Found: C, 39·81; H, 6·24. $C_7H_{13}O_5Cl$ requires C, 39·53; H, 6·16%).

Periodate Oxidation of Fraction (A) (2-Deoxy-2-Chloro α-Methylaltroside)

The oxidation was done according to the standard procedure for such oxidations. 78·4 mg. of the chlorohexoside was taken for oxidation. The uptake of the oxidant was 1·08 mol./mol. Therefore, it contains a pair of contiguous hydroxyl groups.

Periodate Oxidation of Fraction (B) (3-Deoxy-3-Chloro α-Methylglucoside)

The substance (82·0 mg.) was oxidised with sodium periodate. Periodate consumed mol./mol. was 0·03. Hence, it does not contain a pair of adjacent hydroxyl groups.

Periodate Oxidation of the Mixture of Chlorohexosides

The mixture of chlorohexosides (103·7 mg., m.p. 117–122°) was oxidised with sodium periodate. The uptake of the oxidant was 0·25 mol./mol. which indicates that the mixture contains 25% of 2-deoxy-2-chloro α-methyl altroside and 75% of 3-deoxy-3-chloro α-methylglucoside.

Hydrogenation of 3-Deoxy-3-Chloro α-Methylglucoside

3-Deoxy-3-chloro α-methylglucoside (0·422 g.) was dissolved in water (30 c.c.) containing sodium hydroxide (3 c.c., N.), barium sulphate-palladium catalyst (0·6 g., containing about 10% palladium oxide) added and the mixture hydrogenated at 38° for 40 hours at atmospheric pressure, when about 60 c.c. of hydrogen was absorbed. The solution was neutralised with hydrochloric

acid and then evaporated to dryness under reduced pressure (bath temp. 35°). The residue was extracted with absolute alcohol, filtered and evaporated under reduced pressure. The syrupy residue was dried over P_2O_5 in a vacuum desiccator for 24 hours. Yield of the crude syrup = 0·38 g.

The syrup (0·38 g.) was shaken with freshly distilled benzaldehyde (2 c.c.) and fused zinc chloride (0·5 g.) for 18 hours by which time the solution of the syrup was complete. Water and petrol ether (40–60°) were added and the mixture shaken when a white crystalline product separated. This was filtered and washed with petrol ether and finally crystallised from absolute alcohol giving fine crystalline silky needles, m.p. 189–190° (m.p. undepressed when mixed with an authentic specimen of 4: 6-benzylidene-3-deoxy α-methyl glucoside prepared directly from 3-deoxy α-methylglucoside according to the method of Prins (*loc. cit.*). (Found: C, 62·7; H, 6·5. $C_{14}H_{18}O_5$ requires C, 63·1; H, 6·8%.)

Action of Hydrochloric Acid on 2: 3-Anhydro-4: 6-Benzylidene α-Methylmannoside

4: 6-Benzylidene-2: 3-anhydro α-methylmannoside (4 g., prepared according to the method of Robertson and Griffith, *loc. cit.*) was dissolved in acetone (330 c.c.), hydrochloric acid (12 c.c., 2 N.) was added and the solution refluxed on water-bath for 4 hours. It was neutralised with lead carbonate, filtered and the filtrate distilled to remove acetone. The remaining solution was extracted thrice with ethyl ether to remove benzaldehyde and the aqueous solution left behind was evaporated under reduced pressure. The residual syrup was extracted with boiling ethylacetate and the extract evaporated when a colourless syrup, which gave a positive test for halogens, was obtained. All attempts to crystallise it from alcohol, ether, petrol-ether, benzene and their mixtures proved abortive.

Periodate Oxidation of the Mixture of Chlorohexosides

For oxidation experiment, the mixture was dried in vacuum over P_2O_5 for 48 hours. Syrup (0·0728 g.) was oxidised with sodium periodate according to the standard procedure and the uptake of the oxidant was 0·561 mol./mol. It indicates, therefore, that the syrup contains 56·1% of 2-deoxy-2-chloro α-methylglucoside (XLI) and 43·9% of 3-deoxy-3-chloro α-methyl altroside (XLII).

Condensation of Chlorohexosides with Benzaldehyde

The above syrup (2 g.) was shaken with fused zinc chloride (1 g.) and freshly distilled benzaldehyde (7 c.c.) for 6 hours when the syrup completely

went into solution. The reaction mixture was poured in petrol and water when an insoluble reddish syrup separated in the water layer. The water layer was washed with petrol and then extracted with chloroform. The chloroform extract was successively washed with sodium bisulphate, sodium bicarbonate, water and then dried (Na_2SO_4) and filtered. The filtrate was evaporated under diminished pressure when a thick reddish syrup was obtained which could not be crystallised.

4: 6-*Benzylidene-chloro-acetyl α-methyl hexoside*

The benzylidenated syrup (1·5 g.) after drying (P_2O_5) was dissolved in dry pyridine and acetic anhydride (1·2 ml.) was added. The solution was kept at room temperature for four days. It was poured in crushed ice and rubbed with a glass rod when a sticky mass obtained at the outset solidified. The crude product (1 g.) was recrystallised from ethanol when white crystals, m.p. 126°, were obtained. Repeated crystallisation from ethanol and other solvents did not increase the melting point $[\alpha]_D^{27°}+ 96·6°$ ($c = 0·0238$, in chloroform). (Found: C, 56·2; H, 6·1. $C_{16}H_{19}O_{16}Cl$ requires C, 56·0; H, 5·89%).

Attempted Hydrogenation of 4: 6-*Benzylidene-Chloro-Acetyl α-Methylhexoside*

The substance (60 mg.) was dissolved in alcohol (10 c.c.) and poured in a flask containing the catalyst (palladium on barium sulphate, 1·0 g.) in alcohol (20 c.c.) and sodium hydroxide (N., 0·2 c.c.). The flask was shaken in an atmosphere of hydrogen and the hydrogenation continued for 10 hours. The reaction product was filtered, washed with alcohol and the filtrate neutralised with dilute hydrochloric acid. It was evaporated under reduced pressure when a solid product was obtained. It was recrystallised from alcohol, m.p. 120–125°. It also gave the test for halogens.

Action of Hydrobromic Acid on 4: 6-*Benzylidene*-2: 3-*Anhydro α-Methyl mannoside*

Anhydro mannoside (5 g.) was dissolved in acetone (350 c.c.) containing hydrobromic acid (16 c.c., 2 N.) and refluxed for 4 hours on water-bath. The solution was neutralised with lead carbonate, filtered and the residue washed with acetone. The combined filtrate was distilled to remove acetone and the remaining solution was extracted with ether and finally the aqueous solution was evaporated under reduced pressure. The residue, a colourless syrup, was extracted with boiling ethyl acetate. The extract was boiled with charcoal and filtered and the filtrate evaporated under diminished pressure when a colourless syrup was obtained. (Found: C, 30·8;

H, 5·32. $C_7H_{13}O_5Br$ requires C, 32·7; H, 5·1%. $C_7H_{13}O_5Br.H_2O$ requires C, 30·6; H, 5·5%.)

Periodate Oxidation of the Mixture of Bromohexosides

The syrup (0·0643 g.) was oxidised with sodium periodate. The uptake of the oxidant was 0·524 mol./mol. Therefore, the syrup should contain 52·4% of 2-deoxy-2-bromo α-methylglucoside and 47·6% of 3-deoxy-3-bromo α-methyl altroside.

Syrup (3 g., containing the mixture of bromo-hexosides) was shaken with benzaldehyde (15 c.c.) and fused zinc chloride (3 g.) till the syrup completely went into solution. Sodium carbonate (4·5 g.) was added and benzaldehyde removed by steam distillation under reduced pressure. The residue was extracted with chloroform, the chloroform extract evaporated under diminished pressure when a syrup (1·4 g.) was obtained which would not crystallise.

Acetylation of the Benzylidene Bromohexosides

Syrup containing benzylidenated bromo-hexosides (1·4 g.) was dissolved in dry pyridine and acetic anhydride (1 c.c.) was added. The solution was kept at room temperature for three days and then poured in crushed ice when a syrupy mass was obtained. It was extracted with chloroform, the extract washed successively with sodium bisulphate, sodium bicarbonate and water. The solution was dried (Na_2SO_4) and evaporated under reduced pressure when a syrup was obtained which defied all attempts at crystallisation.

A part of this work was carried out by one of us (S. M.) at the University Chemical Laboratory, Cambridge, England, during the year 1946.

The authors wish to thank Professor J. M. Saha, Director, Indian Institute of Sugar Technology, for his kind interest in the work.

SUMMARY

The action of alkaline and acidic reagents and hydrogen on 2:3-anhydro rings in aldohexopyranosides, aldopentopyranosides and aldopentofuranosides has been discussed.

The action of hydrochloric acid on 4:6-benzylidene 2:3-anhydro α-methylalloside has been studied. 2-Deoxy-2-chloro α-methylaltroside and 3-deoxy-3-chloro α-methylglucoside have been obtained. The proportions of these isomers and their structures have been determined by periodate oxidation. The identity of 3-chloro α-methylglucoside has been established by converting it into 4:6-benzylidene-3-deoxy α-methylglucoside. The action of hydrochloric and hydrobromic acids on 4:6-benzylidene-

2:3-anhydro α-methylmannoside has also been studied. The proportions of the halogenohexosides formed as a result of the cleavage of the anhydro ring have been estimated by periodate oxidation. Most of the derivatives of halogenohexosides have been obtained in a syrupy state. Only 4:6-benzylidene-chloro-acetyl α-methylhexoside has been obtained in a crystalline form.

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TEMPERATURE DEPENDENCE OF ULTRASONIC VELOCITY IN LIQUID MIXTURES

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1. Introduction

Rama Rao (1940, 1941) showed that for normal liquids, the quantity $R = \frac{Mv^{1/3}}{d}$ is independent of temperature, M v, and d being respectively the molecular weight, the ultrasonic velocity and the density of the liquid. He further pointed out that R increased proportionately with molecular weight in a homologous series and was hence related to the chemical constitution of the liquid. On the basis of this view, any change in the composition of the molecule such as for example the dissociation of a molecular complex in an associated liquid would show up as a change in R. One way of bringing about this dissociation is by heating the liquid. This was employed by Weissler (1947) and Van Itterbeek (1948) for the determination of the degree of association.

The study of miscible liquid mixtures with particular reference to R is also interesting. A binary mixture may consist of components (*a*) both normal, (*b*) one of them normal and the other associated, (*c*) both associated. In each case R may be studied by varying the concentration as well as the temperature. The ultrasonic velocities in such mixtures at different concentrations have been studied by a number of workers. Using the known densities and assuming a mean molecular weight, R for different concentrations is easily calculated. This has been attempted by Utter and Kling (1948). There is however very little data available regarding the temperature dependence of velocity in such mixtures. The study of R at different temperatures for a few concentrations of mixtures has been attempted in this paper. Employing the experimental set-up already described in a previous paper (1951) the mixtures benzene-carbon tetrachloride, benzene-toluene and ethyl alcohol-benzene are investigated. The results are given in the following tables. In Figs. 1 and 2 are shown the velocity-temperature graphs for the three mixtures. They are drawn to the same scale. It will be seen that all of them are nearly straight lines with negative slopes. The gradients are given in the tables. In the last but one column the values of $\frac{v^{1/3}}{d}$ are shown.

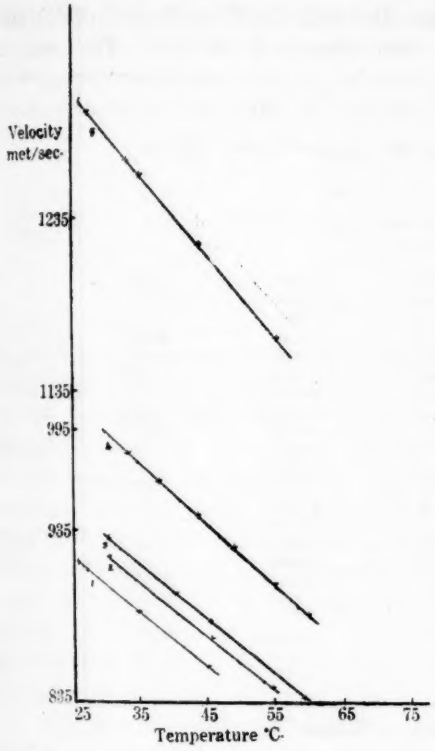

FIG. 1

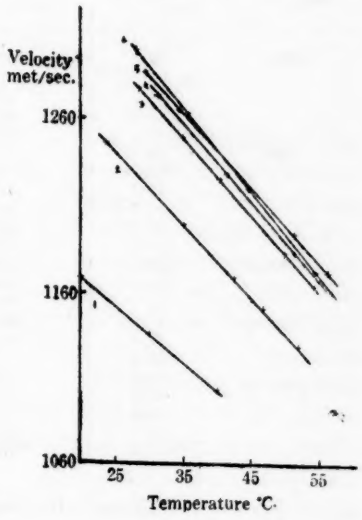

FIG. 2

FIG. 1.—1. CCl_4. 2. C_6H_6-CCl_4·0835. 3. C_6H_6-CCl_4·1596. 4. C_6H_6-CCl_4·3500. 5. C_6H_6.

FIG. 2.—1. C_2H_5OH. 2. C_2H_5·OH-C_6H_6·3290. 3. C_6H_5·CH_3-C_6H_6·4278. 4. C_6H_5·CH_3-C_6H_6·1974. 5. C_6H_5·CH_3. 6. C_6H_6.

2. Experimental Results and Their Discussion

Benzene and carbon tetrachloride are normal liquids. Their molecules are non-polar and the R in each case is independent of temperature. A mixture of these two liquids however, behaves like an associated liquid at every concentration. With increasing temperature, R, which is proportional to $\frac{v^{1/3}}{d}$ shows an increase. For a solution to maintain its R constant it is necessary that the molecular species that compose it should possess the same size, shape and molecular field strength so that the individual molecules are under the same constraints in the solution as in the pure state. In brief the solution has to be ideal. The benzene-carbon tetrachloride mixture is far

from ideal. This mixture has been discussed by Ram Prasad (1942) and the anomalies encountered have been described by him. The mixture benzene-toluene which has been next studied differs from the benzene-carbon tetrachloride mixture in that in the former the molecules of the component

TABLE I. *Benzene-Carbon Tetrachloride Mixture*

Mole fraction Benzene	Temp. °C.	v velocity m./sec.	d density gm./c.c.	$\frac{v^{\frac{1}{3}}}{d}$	Gradient m./sec. degree
·0835	30·5	921·3	1·524	6·385	
	35·0	903·9	1·515	6·382	
	38·5	892·9	1·508	6·384	3·1
	45·4	873·0	1·495	6·396	
	54·5	844·9	1·478	6·396	
·1596	30·1	933·9	1·4690	6·653	
	40·1	900·6	1·4490	6·664	
	44·7	884·3	1·4400	6·665	3·3
	51·8	863·2	1·4263	6·675	
	54·5	853·2	1·4210	6·675	
	59·0	838·1	1·4128	6·674	
·3500	28·5	995·3	1·3425	7·437	
	33·5	980·7	1·3340	7·446	
	37·7	964·7	1·3269	7·445	
	43·5	943·1	1·3165	7·449	3·5
	49·2	925·6	1·3065	7·459	
	54·8	904·7	1·2965	7·459	
	59·3	886·4	1·2885	7·455	
1	27·3	1298	·8706	12·53	
	35·4	1260	·8618	12·53	4·7
	44·0	1218	·8528	12·53	
	54·5	1169	·8413	12·53	

TABLE II. *Benzene-Toluene Mixture*

Mole fraction Benzene	Temp. °C.	v velocity m/sec.	d density gm./c.c.	$\frac{v^{\frac{1}{3}}}{d}$	Gradient m./sec. degree
0	28·6	1287	·8563	12·70	
	34·0	1264	·8508	12·70	
	44·5	1221	·8408	12·71	4·1
	50·6	1194	·8348	12·70	
	56·8	1170	·8290	12·71	
·1974	28·2	1283	·8594	12·64	
	31·5	1270	·8558	12·65	
	41·0	1228	·8460	12·65	4·3
	46·0	1206	·8410	12·65	
	51·5	1182	·8366	12·64	
	57·2	1158	·8296	12·65	
·4278	27·8	1279	·8625	12·58	
	34·7	1248	·8550	12·59	
	40·2	1224	·8496	12·59	4·25
	50·0	1182	·8396	12·59	
	54·8	1163	·8348	12·59	
	59·3	1145	·8302	12·60	

TABLE III. *Ethyl Alcohol-Benzene Mixture*

Mole fraction Benzene	Temp. °C.	v velocity m/sec.	d density gm./c.c.	$\frac{v^{\frac{1}{3}}}{d}$	Gradient m/sec. degree
·3290	23·5	1245	·8526	12·62	
	34·5	1199	·8417	12·62	4·1
	42·2	1168	·8339	12·63	
	46·5	1150	·8292	12·63	
	53·5	1120	·8217	12·64	

liquids are very much similar, whereas in the latter they differ widely. The graphs show this in a striking manner, as may be seen from the closeness of the benzene-toluene lines. For two concentrations, the proportionate R values are given. It is clear that the variation with temperature, if any, is very much less for this mixture than for the benzene-carbon tetrachloride solution for the same concentrations. Young and Fortey (1903) have studied the mixture and reported a small deviation from the laws of ideal solution. Incidentally it is interesting to note that whereas the temperature coefficients of velocity of benzene and carbon tetrachloride are respectively 4·7 and 3·1 metres per sec. per degree, the difference is very much less for benzene and toluene; toluene having a coefficient of velocity of 4·1 metres per sec. per degree.

The mixture ethyl alcohol-benzene has been discussed by Ram Prasad (1942). It is well known that ethyl alcohol is a highly associated liquid. Its R changes from 614 at 20° C. to 616 at 50° C. The corresponding values for $\frac{v^{1/3}}{d}$ being 13·35 and 13·39. These may be compared with the values given in the tables for the mixture. The variation is small indicating a smaller association due to the presence of benzene molecules.

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SURFACE CHEMISTRY OF CONGORUBIN SOLUTIONS*

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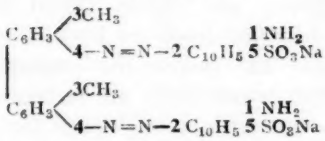
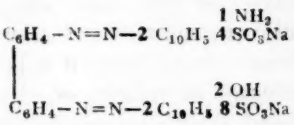
While studying the effect of the electrical field on Tyndall scattering in aqueous solutions of dyes, it was noticed that the electric field affected Tyndall scattering in the case of benzopurpurin-6 B but not with congorubin. The difference in behaviour is rather surprising, as the two dyes appear to be similar in structure, each molecule having four hydrophilic groups as shown below:—

Benzopurpurin



Congorubin



Evidently there seems to be some difference in spacial structure between the two dyes. To throw light on the phenomenon, the surface properties of congorubin solutions were studied. Some aspects of the surface behaviour of benzopurpurin have been studied by Doss.[1]

Experimental

The congorubin used in this investigation was a Kahlbaum product and was purified by the sodium acetate method described by Robinson.[2]

The surface pressures were measured by the modified film technique described by Doss.[1] Measurements were made at a pressure of 0·9 dyne per cm. as the film would collapse at higher pressures.

For the study of the rate of accumulation, various concentrations (0·1% to 0·01%) of congorubin were employed. The effect of pH on the rate of accumulation was studied using 0·0125% solution of the dye. The pH of this solution was lowered by the addition of dilute acetic acid. The influence of foreign substances such as saccharose, amyl alcohol, Nekal Bx, sodium

* Part of the thesis submitted by R. S. Subrahmanya for the M.Sc. degree of the Mysore University.

stearate and valeric acid on accumulation was also studied. The results are given in Table I. A glass electrode was employed to measure the pH of solutions.

TABLE I

Sl. No.	% Weight of congo-rubin in solution	pH	Foreign substance	Fraction of the surface occupied in				
				1 min.	2 min.	4 min.	8 min.	16 min.
1	0·10	6·8	..	0·101	0·122	0·158	0·216	0·320
2	0·05	,,	..	0·061	0·079	0·100	0·146	0·186
3	0·025	,,	..	0·043	0·057	0·079	0·102	0·145
4	0·0125	,,	..	0·031	0·043	0·055	0·082	0·108
5	0·0125	4·7	Acetic acid to adjust pH	0·070	0·086	0·100	0·140	0·180
6	0·05	6·8	3% saccharose	0·037	0·043	0·059	0·091	0·128
7	0·10	,,	0·17% amyl alcohol	0·070	0·102	0·141	0·201	0·310
8	0·10	,,	0·002% Nekal Bx	0·572	0·703	0·902	..	..
9	Nil	,,	0·002% Nekal Bx	0·137	0·154	0·174	..	..

Of the several methods available for the study of surface tension, only a few can be employed for investigating the variation of surface tension with time. As pointed out by Doss,[3] the dynamic methods are unsuitable for this purpose. Venkatachala[4] has used the ring method for the study of Nekal Bx solutions. When this method is employed for congorubin solutions, the surface tension measured corresponds only to that of water. Evidently this technique is inapplicable to the study of congorubin solutions, wherein condensed films are formed and the attainment of equilibrium is therefore exceedingly slow, while with Nekal Bx the film produced is of the gaseous type and the rate of accumulation is very high. The trough method was found to be suitable for the study of congorubin solutions. The strength of the rubin solution in this case was 0·2%. The results are represented in Graphs 1 and 2.

The area of cross-section of congorubin was determined in the following way. A paraffined barrier was kept on the trough, almost touching the float. For forming the fresh surface, the barrier was moved away from the float. The fresh surface thus formed between the barrier and the float was allowed to age for about two minutes, during which period, the rate of accumulation of the dye was relatively high. Then, the dye that had collected on the surface was enclosed between the barrier and the float by moving the barrier towards the float. With another barrier, a fresh surface was allowed to form, by repeating the above operation starting from the first barrier, instead of the float. This surface also was allowed to age for 2 minutes, and the accumulated dye pushed towards the float, after removing

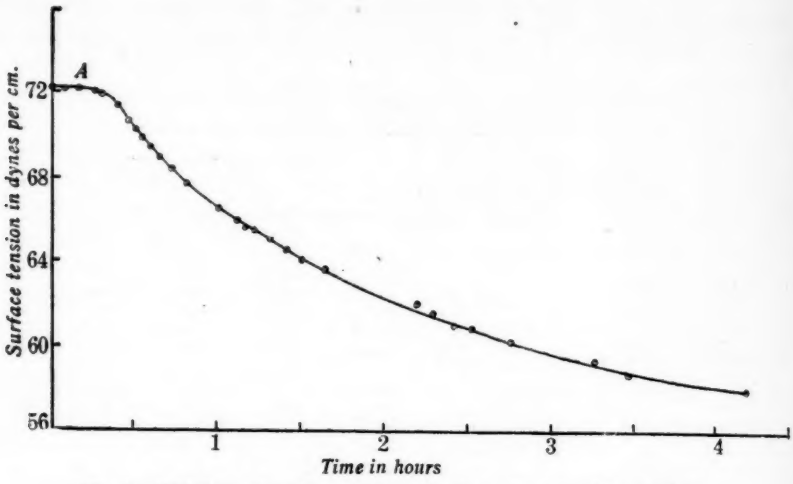


FIG. 1. Variation of surface tension with time of congorubin solution

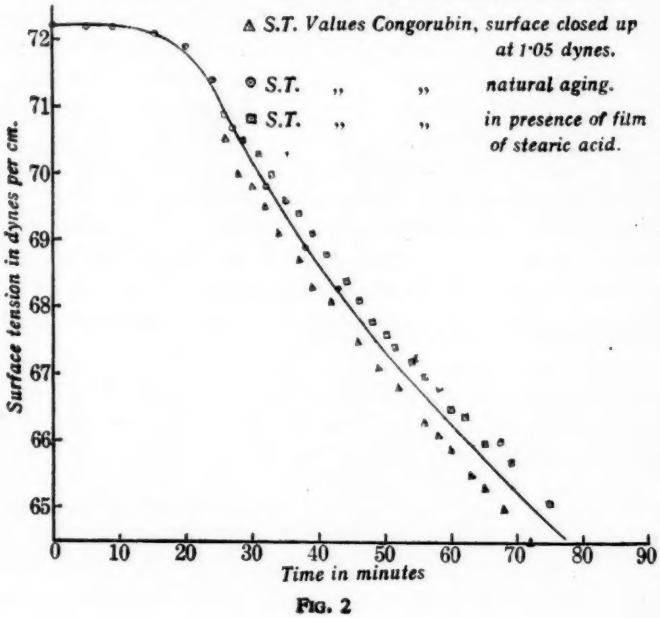


FIG. 2

the first barrier. This technique was repeated several times, so that about three-fourths of the surface of the trough was covered with the dye. In this way, the total time taken to form the film was reduced to about 20 minutes, while accumulation would normally have required several hours. The congorubin film trapped between the float and the barrier was allowed to age for about 40 minutes. This was found to be quite adequate for the attainment of equilibrium. The film was then swept, collected in a well at the end of the trough, and weighed.[5] Its concentration was determined by comparing its colour with that of a standard congorubin solution in a colorimeter.

Very dilute solutions (0·0002 M) were employed in the trough. This secured adequate accuracy in the subsequent colour comparison. The area of cross-section of the congorubin molecule is equal to

$$\frac{A.M.}{W(x_2 - x_1)N},$$

where x_1 = No of g. of dye in 1 g. of the origidal solution.

x_2 = No. of g. of dye in 1 g. of the sweepings.

W = Wt. of the sweepings.

M = Molecular weight of the dye.

N = Avogadro Number.

A = Total area occupied by the film.

The cross-section of the rubin molecule obtained by this method was

$$42 \times 10^{-16} \text{ sq. cm.}$$

Discussion

Accumulation of congorubin from aqueous solutions

The data presented in the table indicate that congorubin molecules accumulate slowly at a water surface. Increase in concentration enhances the rate of accumulation. At lower concentrations (0·05% and below), for a given accumulation, the time is inversely proportional to concentration. Thus, at a concentration of 0·05%, two minutes are required to increase the surface covered from 7·9 to 10% while at 0·025% the time required is nearly four minutes to bring about the same effect. When the equation $\frac{dx}{dt} = K_1 c (1 - x)$ is applied to the values obtained, K_1 is found to be inconstant and to diminish with time. It is of interest to note, that when the pH of the solution was lowered by the addition of acetic acid, accumulation during the first minute

was nearly doubled (compare experiments 4 and 5). When lowering in pH of the same order was effected by the addition of valeric acid (instead of acetic acid) the effect was very slight, the accumulation in the acidified solution being 0·07 at the end of the first minute while in absence of the acid the value was 0·061. The addition of amyl alcohol or of sugar decreased the accumulation of the dye while Nekal Bx increased it. Sodium stearate, on the other hand, had very little effect.

Several theories,[6, 7, 8, 9, 10] have been put forward to account for the phenomenon of slow accumulation. The most satisfactory explanation seems to be the one proposed by Doss.[10] He suggests that the orientation of the molecules of the dye and the formation of an electrical double layer are responsible for slow accumulation. In the case of condensed films, slow accumulation is mainly due to the orientation effect. The electrical double layer is responsible for slowing down the accumulation in gaseous films.

Examination of the F–A relationship indicates that congorubin forms a condensed film at the surface of water. Owing to the four hydrophilic groups in its molecule, the dye is quite soluble in water. At each end of the hydrophobic chain the dye has two hydrophilic groups. When a molecule of the dye comes up to the air-solution interface the molecule may possibly orientate itself in two ways: (1) The molecule may bind itself in such a way that all four hydrophilic groups are anchored in the aqueous phase. (2) Only one hydrophilic end may be in the water and the other, out in the air. In the first case the molecule is very likely to be dragged into solution because of the predominant pull of the hydrophilic groups and there will thus be no accumulation at all at the surface. In the second way however, there will be an accumulation of the dye at the air-solution interface to form a condensed film. But, only molecules of the dye that have high translational energy can come up and orientate in this fashion. The number of such molecules must obviously be very small. This accounts for the slow accumulation.

It has been pointed out already, that in the case of congorubin, accumulation falls off with time. This effect seems to be due to the concentration of negative charges at the interface, due to the ionised hydrophilic groups of the dye anchored in the solution. This charged layer makes it increasingly difficult for molecules of the dye to come up and accumulate at the interface.

The effect of acetic acid on the accumulation can easily be accounted for. The lowering of pH brought about by the addition of the acid decreases the negative charge at the interface. It is noticed that valeric acid has far

less effect than acetic acid on the accumulation. This seems to be due to the formation of a complex between the valeric acid and the dye. Owing to the formation of this complex, the hydrophilic character of the dye is enhanced and it becomes more difficult for the dye molecule to take up the particular position resulting in accumulation. It may be pointed out that amyl alcohol, which does not affect the pH, also causes a diminution in accumulation. This is probably due to complex formation resulting in an increase in hydrophilic character, as with valeric acid. The decrease noticed with saccharose can be accounted for on the same basis.

When Nekal Bx is mixed with congorubin the combined accumulation is far greater than the sum of the accumulation of each component, as can be seen from Table I. Nekal Bx has a large hydrophobic group, and the complex of Nekal Bx with the congorubin, compared with the dye, will be more hydrophobic in character. The increase in hydrophobic character facilitates accumulation. In the case of sodium stearate however, the rate of accumulation is practically unaffected. The fraction of the surface covered with sodium stearate (0·0002%) in 1 min. was 0·150 and with congorubin 0·061. A mixture of rubin and stearate gave a value of 0·216, which is practically the same as the sum of the two individual accumulations, *viz.* (0·211). As has been shown later in this paper, the formation of a film of congorubin is not affected by the presence of stearic acid at the surface at which the film of the dye is produced.

Variation of surface tension with time

The variation of surface tension with time is plotted in Graph 1. It is seen that the surface tension of the rubin solution is practically the same as that of pure water during the first fifteen minutes. At this stage however (point A), a distinct fall in surface tension is noticed. This fall is rapid at first but slows down with time. These observations can be accounted for, on the basis of accumulation of congorubin. Up to the point A the congorubin molecules that come up to the surface, form only patches or islands of condensed film. At A however, there is practically complete union of the islands to form a compact film. Thereafter, the molecules of the dye that come up to the surface increase the surface pressure and cause a lowering of surface tension. This effect is marked in the beginning but progressively falls off. This is to be expected, as the rate of accumulation of rubin molecules rapidly diminishes with time.

In Graph 2 is illustrated the accumulation of congorubin under different experimental conditions: (1) natural ageing, (2) ageing when the surface is closed up at a pressure of 1·05 dynes, (3) ageing in presence of a film of

stearic acid. From Graph 1, it will be noticed that a period of 25 minutes is necessary to develop a surface pressure of 1·05 dynes. This period has been taken into account in determining the points for Graph 2. The three curves lie very close to one another and it may therefore be concluded that once a compact film has been formed on the surface of the congorubin solution, the lowering of surface tension due to further accumulation is the same in all the three cases. The presence of stearic acid does not affect the accumulation of congorubin. The area of 42×10^{-16} sq. cm. for the cross-section of the congorubin molecule obtained by the trough method indicates that the molecule of the dye orients itself on the surface of water with one end having two hydrophilic groups of the hydrophobic chain away from the water, while the other two hydrophilic groups of the chain are anchored in water.

SUMMARY

1. The rate of accumulation of congorubin molecules at an air-water interface has been investigated under varying conditions of concentration and pH. The rate of accumulation increases with the increase in the concentration of the congorubin solution. The rate is also enhanced by an increase in the hydrogen ion concentration. The results are explained on the basis of activated accumulation.

2. The effect of (*a*) acetic acid, (*b*) valeric acid, (*c*) saccharose, (*d*) amyl alcohol, (*e*) sodium stearate and (*f*) Nekal Bx, on the accumulation has been investigated. With (*b*), (*c*) and (*d*) a fall in accumulation is noticed while in (*a*) and (*f*) there is an increase. In (*e*) on the other hand, no change is observed. These results are explained on the basis of orientation of the molecule of the dye. Formation of a complex by the congorubin with valeric acid, saccharose, amyl alcohol and Nekal Bx is responsible for the departure from the normal behaviour of the dye.

3. The trough method is employed to study the variation of surface tension with time. The surface tension shows no variation during the first 15 minutes, but, after this period suffers a lowering with time. The lowering becomes less as ageing proceeds and tends to reach an equilibrium value.

4. The accumulation of congorubin is not affected by the presence of a stearic acid film on the surface of congorubin solution.

5. The area of cross-section of the congorubin molecule has been determined by the trough method. This value supports the view that there is orientation of the congorubin molecule at the air-water interface.

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COMPONENTS OF THE BARK OF *PRUNUS PUDDUM**

Part II. Padmakastin and Padmakastein

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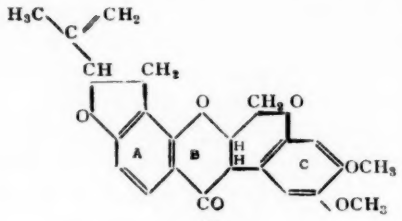
IN the complex molecular structure of rotenone (I) and other rotenoids in which there are five rings, the essential core would appear to be the isoflavanone structure (A, B and C). Though many isoflavones have been obtained from plant sources and by synthesis no simple dihydroderivatives (isoflavanones) have so far been recorded in the literature and characterised. In this paper are described the isolation of the first member representing this core of rotenoids. Padmakastin is the glycoside and Padmakastein (II) is the aglucone newly obtained from the bark of *Prunus puddum* and belonging to the abovementioned new type. The new names are derived from the Sanskrit name of this plant, *Padma kashta*. The more convenient names like puddumetin and prunusetin derived from the botanical name of the plant have already been employed[1] for other substances obtained from it though they are not new and have other names. Their use in the new context will create confusion.



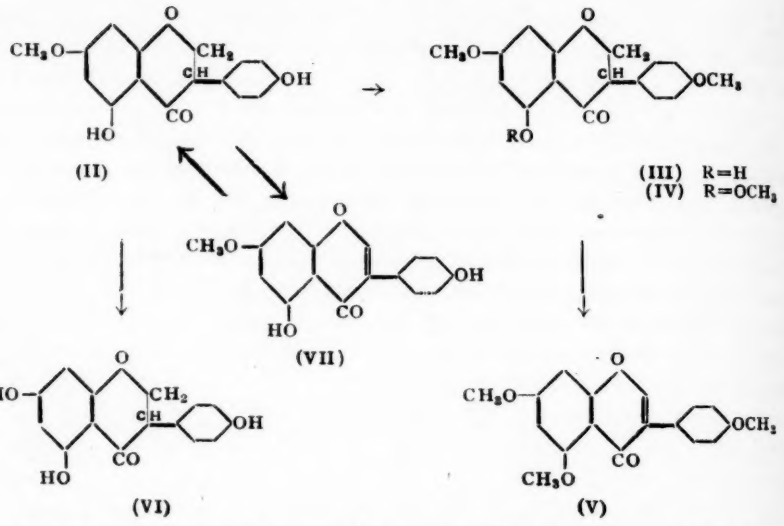
(I)

In our efforts to get prunetin, sakuranetin and genkwanin from the bark of *Prunus puddum*[2] we noticed there was considerable variation in the composition of samples obtained in various seasons. Two points are noteworthy. Samples collected in late summer (reported in our earlier publication[2]) contained very small quantities of glycosides whereas those obtained in late winter gave considerable yields of the glycosidic fraction. Further in the summer sample along with sakuranetin there was found another

* An earlier paper[2] on this subject entitled "A Note on the Components of the Bark of *Prunus puddum*" is considered to be Part I.

substance (not reported earlier) in very small quantity. This was present in larger quantities in the winter sample. Actually it has been possible to isolate this substance in adequate yield for a chemical examination from a sample of the bark obtained in February 1951. It is a non-glycosidic body identical with the aglucone of the new glycoside mentioned below. Further the glycoside fraction could be separated into two; the more soluble portion contained largely sakuranin whereas the less soluble fraction consisted of a new glycoside padmakastin. The aglucone padmakastein (II) has nothing in common with a substance reported by Chakravarti, Kundu and Ghosh[3] as found along with sakuranetin in the sample investigated by them. There is difference in melting point, colour reaction and composition. It is definite therefore that the new glycoside and its aglucone are now reported for the first time.

Padmakastein (II) has the molecular formula $C_{16}H_{14}O_5$, contains one methoxyl group and yields a diacetate. It undergoes partial methylation to form a monomethyl ether (III) and complete methylation to a dimethyl ether (IV). This along with the fact that it gives a prominent colour with ferric chloride would indicate the presence of a hydroxyl group situated ortho to a carbonyl group. The substance does not give the usual flavone or flavanone colour reaction with magnesium and hydrochloric acid but gives a green colour with nitric acid which slowly changes to red. This colour reaction is characteristic of certain hydroxy acetophenone derivatives (methyl ethers) and also of flavanone methyl ethers.[4] The red colour changes to green on addition of ammonia. This is positive Durham test. The sulphuric acid solution is colourless and on adding a few drops of conc. nitric acid develops a feeble yellowish red colour. Another significant feature is the capacity of the substance to reduce iodine. These reactions gave indications that padmakastein is in fact an isoflavanone having the essential features of the rotenone group. There seems to be no doubt that this part is the one responsible for the positive Durham test. The isoflavanone constitution has been confirmed by the dehydrogenation of padmakastein dimethyl ether (IV) with selenium dioxide in amyl alcohol solution yielding genistein trimethyl ether (V) which on demethylation yields genistein. Thus it is definite that padmakastein is a dihydrogenistein-monomethyl ether. The location of the methoxyl group became clear from the following considerations. The substance is insoluble in aqueous sodium carbonate but on demethylation with aluminium chloride it yields the nor compound (VI) which is soluble in this reagent. From considerations already explained elsewhere[5] it follows that the methoxyl group is located in the 7-position and that padmakastein is dihydroprunetin (II).



It has also been possible to reduce prunetin (VII) by means of sodium *meta*-bisulphite to the dihydro derivative which is found to be identical with the new aglucone padmakastein. The above reducing agent seems to provide a convenient method for the reduction of the pyrone ring to the corresponding pyranone. In this connection may be mentioned the recent report of Robertson, *et al.*[6] who studied catalytic hydrogenation of santal methyl ether and were able to obtain a non-ketonic product which they considered to be produced by the reduction of the CO group in addition to ethylenic double bond. Hence the *meta*-bisulphite method, has the distinct advantage of reduction of the ethylenic double bond only.

Another reaction which we have successfully carried out should also be mentioned. Earlier experiments on the use of selenium dioxide for dehydrogenation of flavanones or chalkones were found to proceed satisfactorily only for producing fully methylated flavones.[7] This method could not be successfully used for the preparation of partial methyl ethers of flavones.[3] It is now found that the acetate of padmakastein undergoes dehydrogenation with selenium dioxide in acetic anhydride solution very satisfactorily to yield prunetin acetate which on deacetylation yields prunetin (VII). This procedure is capable of being adopted in analogous cases of flavanone partial methyl ethers.

The association of sakuranetin and padmakastein is parallel to that of genkwanin and prunetin. As for the glycosidic fraction it seems to consist

entirely of the reduced compounds sakuranin and padmakastin. The nature of sugar and its position in padmakastin will be discussed later.

Experimental

Extraction.—The sun-dried bark of *Prunus puddum* (3 kg.) was powdered and extracted twice with cold alcohol by percolation. Each extraction was carried out for 48 hours. The deep red alcoholic extract was concentrated to about 500 c.c. and allowed to stand; after two days a yellow sticky solid separated out. This was filtered and the residue marked (fraction A). The alcoholic filtrate was then treated with excess of ether (1 litre) till it formed two layers and left in the ice chest. After a few days an almost colourless solid (very pale yellow) separated out. It was filtered, washed with ether and marked (fraction B). After separating the ether layer the mother-liquor was twice extracted with hot benzene, and the ether and benzene fractions combined (fraction C). The residual viscous liquid (marked D) was directly hydrolysed with aqueous alcoholic sulphuric acid (7%).

Fraction A (*Genkwanin, prunetin and padmakastein*).—The solid was boiled with benzene (10 c.c.) twice and filtered. The residue was then boiled with excess of alcohol (60 c.c.) and the clear solution filtered. On cooling it slowly deposited a yellow solid which when recrystallised from alcohol melted at 285° and was identical with genkwanin. When the alcoholic filtrate was concentrated (to 40 c.c.) and then cooled a small quantity of a pale yellow solid separated. After two crystallisations the compound melted at 236–8° and was found to be identical with prunetin. On completely distilling off alcohol from the mother-liquor an almost colourless solid separated. After two crystallisations from alcohol it melted between 225–30° and further crystallisations did not improve the melting point. The product was acetylated with acetic anhydride and pyridine and the dry acetate crystallised from ethyl acetate. The fraction that separated on cooling melted at 218–20°. A recrystallisation from ethyl acetate yielded glistening colourless rhombic prisms having the same melting point. It slowly developed a green colour with conc. nitric acid (Found: C, 65·3; H, 5·0; $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9%). By further concentration of the ethyl acetate solution and crystallisation of the product a little more of the above acetate was obtained. Complete removal of the ethyl acetate gave some impure substance melting between 190–95° and was not worked up.

When the acetate was deacetylated by heating in alcohol (10 c.c.) with conc. hydrochloric acid (10 c.c.) on a water-bath for 15 minutes the hydroxy compound separated as colourless needles. The mixture was diluted with water (20 c.c.) and the solid product filtered and washed with water. On

crystallising from alcohol the compound separated as colourless rectangular plates and prisms melting at 236–8°; a mixed m.p. with prunetin was depressed. It gave a green colour with conc. nitric acid; the colour changed red on standing, but the green colour was again produced on adding strong ammonia. The substance formed a reddish violet colour with ferric chloride. It was insoluble in aqueous sodium carbonate but dissolved readily in aqueous sodium hydroxide giving a yellow solution. An alcoholic solution of iodine and sodium acetate was readily decolourised when added to an alcoholic solution of this substance. It gave no colour with magnesium and hydrochloric acid. It differed in its properties and reactions from the other components of the bark and is given the name padmakastein (Found: C, 66·7; H, 5·0; $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%). Yield, genkwanin, 0·5 g.; prunetin, 1 g. and padmakastein, 1 g.

Fraction B (*padmakastin and sakuranin*).—The crude glycoside was boiled with alcohol (15 c.c.) twice and filtered when only part of it went into solution. The residue was repeatedly crystallised from alcohol and then it melted at 225–7° (padmakastin). It gave a red colour with ferric chloride and a green colour with conc. nitric acid. On hydrolysis with 7% aqueous alcoholic sulphuric acid it gave padmakastein. When the original alcoholic solution was cooled it yielded some more of the new glycoside. On concentrating it further and crystallising the product from aqueous alcohol a colourless crystalline solid was obtained which melted at 210–12° and agreed in all its properties with the description of sakuranin. Yield: new glycoside (padmakastin), 2·0 g. and sakuranin, 1 g.

Fraction C.—This was distilled to remove benzene and worked up as described earlier.[2] Yield of sakuranetin, 3 g.

Fraction D.—The viscous liquid (marked D) was diluted with water (400 c.c.), treated with conc. sulphuric acid (16 c.c.) and the solution refluxed for 2 hours. It was then cooled and left in the refrigerator for 24 hours. The brown solid that separated was filtered; it was boiled twice with benzene (20 c.c.) to remove sakuranetin and filtered. The residue on crystallisation from alcohol melted at 234–36°. It was directly acetylated and the acetate crystallised from dry ethyl acetate from which it came out as colourless rhombic prisms melting at 218–20°. It gave a green colour with conc. nitric acid. On deacetylation with alcoholic hydrochloric acid it gave padmakastein which crystallised from alcohol as colourless rectangular prisms and plates melting at 236–8°. Both the acetate and the hydroxy compound were identical with the samples obtained from the fraction A. Yield of padmakastein, 2·5 g.

Partial methylation (*padmakastein monomethyl ether*) (*III*)

Padmakastein (0·3 g.) was refluxed in acetone (30 c.c.) solution with dimethylsulphate (0·15 c.c.) and anhydrous potassium carbonate (0·5 g.) for 6 hours. On filtering and distilling off acetone from the filtrate a pale yellow solid separated. It crystallised from alcohol as colourless stout prisms melting at 131–32°. It gave a red colour with ferric chloride in alcohol and a green colour with conc. nitric acid (Found: C, 68·4; H, 5·4; $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·3%).

Complete methylation (*padmakastein dimethyl ether*) (*IV*)

Padmakastein (0·5 g.) was refluxed for 24 hours in acetone solution with excess dimethyl sulphate (0·7 c.c.) and anhydrous potassium carbonate (1·5 g.). Acetone was then distilled off, water added to the residue and the solid filtered. The methyl ether crystallised from alcohol as colourless thin leaf-like crystals melting at 146–7°. It gave no colour with ferric chloride and was insoluble in aqueous alkali. It developed a green colour with conc. nitric acid (Found: C, 68·4; H, 5·5; $C_{18}H_{18}O_5$ requires C, 68·8; H, 5·7%).

Demethylation with anhydrous aluminium chloride (*nor-padmakastein*) (*VI*)

Padmakastein (80 mg.) was heated with anhydrous aluminium chloride (0·5 g.) in benzene (10 c.c.) for 2 hours. Benzene was then removed by evaporation and the organo metallic complex decomposed with ice and hydrochloric acid. The solid that separated was filtered, washed with dilute hydrochloric acid and water. It was then dissolved in aqueous sodium carbonate, the solution filtered and the clear filtrate acidified. The colourless product was filtered, washed with water and crystallised from alcohol when it came out as colourless short prisms melting at 270–2°. It gave a reddish violet colour with ferric chloride and was easily soluble in aqueous sodium carbonate (Found: C, 63·6; H, 4·5; loss on drying *in vacuo* at 110°, 3·1; $C_{15}H_{12}O_5$, $\frac{1}{2}$ H_2O requires C, 64·0; H, 4·6; loss on drying 3·2%).

Selenium dioxide oxidation (*O-trimethyl genistein*) (*V*)

Padmakastein dimethyl ether (0·2 g.) was dissolved in amyl alcohol (10 c.c.), selenium dioxide (0·25 g.) added and the mixture heated at 140° for 12 hours. Metallic selenium that was formed in the reaction was then filtered off and from the filtrate amyl alcohol was removed by distillation on a water-bath under reduced pressure. The last traces of amyl alcohol were removed by passing a current of steam. The solid residue was filtered and crystallised from alcohol. It was obtained as colourless needles melting

at 161–2° and was identical with genistein trimethyl ether, the mixed melting point with an authentic sample being undepressed. Yield, 0·12 g.

It was demethylated by heating with anhydrous aluminium chloride in benzene solution. The product which crystallised from aqueous alcohol as colourless tiny prisms melted at 290° and was identical with genistein the mixed melting point being undepressed.

Selenium dioxide oxidation of padmakastein acetate (prunetin acetate)

A mixture of padmakastein acetate (0·2 g.) and selenium dioxide (0·2 g.) in acetic anhydride (7 c.c.) was heated in an oil-bath at 140° for 12 hours. Metallic selenium was filtered off and the acetic anhydride solution poured into ice water. The colurless solid that separated was filtered and washed with water. After drying it was crystallised first from absolute alcohol and again from ethyl acetate when it separated as small rectangular prisms and needles melting at 222–4°. A mixed melting point with an authentic sample of prunetin acetate did not show any depression. Deacetylation of this acetate gave prunetin (VII) (m.p. and mixed m.p. 238–40°).

Reduction of prunetin (VII) to padmakastein (II)

A solution of prunetin (0·5 g.) in aqueous sodium hydroxide (2·5 g. in 50 c.c. of water) was treated with solid sodium *meta*-bisulphite (5 g.) in small quantities with boiling. After the addition was over the solution was boiled for further 5 minutes. It was then cooled in ice and acidified with hydrochloric acid. The colourless solid that separated was filtered, washed with water and crystallised from alcohol. The product separated as colourless rectangular plates and prisms melting at 236–8° with sintering at about 228°. It gave a green colour with conc. nitric acid and decolourised iodine and sodium acetate in alcohol. It was directly acetylated by heating with acetic anhydride and pyridine. The acetate crystallised from ethyl acetate as colourless glistening rectangular plates and prisms melting at 218–20° and mixture with padmakastein acetate described earlier melted at the same temperature.

SUMMARY

Two new substances, padmakastin, a glycoside and padmakastein, the corresponding aglucone have been isolated from the bark of *Prunus puddum*. The properties and reactions of padmakastein indicate that it is dihydroprunetin. This has been confirmed by its oxidation to prunetin and by its preparation from prunetin by reduction. Padmakastein is the first

isoflavanone to be isolated and it gives the well-known Durham test characteristic of rotenoids.

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